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Effect of spatial visual cue proximity and thalamic lesions on performance of rats on a cheeseboard maze task

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Abbreviations

ACQ	standard acquisition trials
AD	anterodorsal thalamic nucleus
AM	anteromedial thalamic nucleus
ANOVA	analysis of variance
AT	anterior thalamic nuclei
AV	anteroventral thalamic nucleus
B-L	bregma to lambda measurements
Contra	contralateral
D-V	dorsal to ventral measurements
DX-PX	distal then proximal task order
FX	fornix
HPC	hippocampus
ILN	intralaminar thalamic nuclei
IP	intraperitoneal
Ipsi	ipsilateral
LD	laterodorsal thalamic nuclei
LDDM	laterodorsal dorsomedial thalamic nucleus
LDVL	laterodorsal ventrolateral thalamic nucleus
LP	lateroposterior thalamic nuclei
LT	lateral thalamic nuclei
MB	mammillary bodies
M-L	medial to lateral measurements
MRI	magnetic resonance imaging
MT	medial thalamic nuclei
MTL	medial temporal lobe
NIH	National Institutes of Health
NMDA	*N*-methyl-D-aspartate
P1	probe 1
P2	probe 2
P2a	probe 2 (allocentric measure)
P2e	probe 2 (egocentric measure)
P3	probe 3
pACQ	acquisition trials that occurred during probe weeks
PC	parietal cortex
PH	parahippocampal cortex
PRC	perirhinal cortex
PX-DX	proximal then distal task order
RF	radio frequency
rH	relative humidity
RSP	retrosplenial cortex
sAT	sham anterior thalamic lesion
SEM	standard error of the mean
sLD	sham laterodorsal thalamic lesion

Abstract

Episodic memory is processed by the extended hippocampal system, and pathology or injury to individual components of this system can result in deficits in spatial learning and memory (Aggleton & Brown, 1999). Extensive research regarding spatial memory has been carried out on the anterior thalamic nuclei, a component of the extended hippocampal system, but the contribution of the laterodorsal thalamic nuclei, an adjacent structure with similar neural connections, is less clear. The purpose of the present study was to compare the effects of selective anterior thalamic nuclei lesions (AT) with selective laterodorsal thalamic nuclei lesions (LD) in a novel land-based spatial reference memory task. This assessed the use of proximal and distal visual cues on the propensity to use allocentric or egocentric navigation strategies to locate a specific place in space, as well as the temporal evolution of these navigation strategies. AT lesion impairments were observed in the acquisition trials in both proximal and distal cue conditions. LD lesion rats were unimpaired in the acquisition trials in both visual cue conditions. Across the probe trials, lesion effects were not observed when tested for general navigation, egocentric or allocentric strategies, and there was no clear improvement in performance over the four weeks of probe trials. However, performance was consistently poorer for all groups when proximal cues facilitated navigation compared to distal cues. Performance differences related to cue proximity may reflect the influence of motion parallax, the perceived displacement rate of visual cues. The absence of lesion effects across probes were thought to be due to the preferential use of cued navigation, which was reliant on a single salient beacon, and the lack of integration between cued and place navigation, which was reliant on the formation of a spatial representation.

1. Introduction

1.1. General

The ability to form new memories is critical to daily functioning. For people who suffer from disorders like dementia, thalamic strokes and Korsakoff's syndrome, this function is impaired, with anterograde amnesia a common symptom across each of these disorders. The emphasis of anterograde amnesia lies in the inability to recall recently formed memories, in particular, episodic memories. Episodic memory refers to events that are personally experienced and incorporates spatial (what and where) and temporal (when) information (Dere, Kart-Teke, Huston, & De Souza Silva, 2006; Fleischman & Gabrieli, 1999; Tulving, 2001). This contrasts semantic memory, which involves facts and rules. For example, although one could recall that the composer Wolfgang Amadeus Mozart was born on the 27th January 1756 in Salzburg, it is a semantic rather than episodic memory as it lacks personal experience, despite it containing spatial and temporal information.

The diffuse and uncertain pathology of human amnesia makes it difficult to investigate the contribution of specific structures and the neural connectivity involved in episodic memory in isolation. However, anatomical data from human cases of amnesia provide a basis to develop animal models of amnesia (Corkin, 2002; Corkin, Amaral, González, Johnson, & Hyman, 1997). An exemplary case of a human study leading to animal models of learning and memory, was the patient H.M. To ameliorate his severe epilepsy, H.M. had a large portion of his medial temporal lobe removed bilaterally, including the hippocampus, which resulted in severe episodic memory loss (Scoville & Milner, 1957). One of the tests of H.M.'s cognitive function examined navigation ability. This is of interest because it encompassed two facets of episodic memory: spatial and temporal information. Spatial navigation is a complex skill that humans undertake on a daily basis as it enables way-finding, and orientation within the environment. Interestingly, H.M. was eventually able to draw the floor plan of the house he lived in post-surgery, but

his performance on nearly all spatial tests within the lab were consistently poor (Corkin, 2002). It is possible that his ability to draw the floor plan came from his intact procedural skills and long-term repetition of walking through the house. Other brain regions may process information that has become habitual. These differences in neurological and behavioural findings instigated an extensive number of studies on the role of the hippocampus and the formation of episodic memories (Aggleton & Brown, 1999; Clearwater & Bilkey, 2011; Correll & Scoville, 1965; Fouquet, Tobin, & Rondi-Reig, 2010; Gilbert, Kesner, & DeCoteau, 1998; Mair, Burk, & Porter, 1998; Reece & Harris, 1996; Save & Poucet, 2000).

The properties of episodic memory were previously thought to be unique to humans because they require the ability to explicitly recall personal experiences using self-perspective (perception of oneself relative to others), self-awareness, and the capacity to instinctively sense time (Dere, et al., 2006). It was believed that because animals are not verbal, they are not capable of consciously recollecting experiences, nor are they able to consciously perceive and use temporal information. However, Dere and colleagues (2006) suggest that it is beneficial for animals to possess episodic memories to enable foraging, by recalling where and when food is available, and to avoid possible future encounters with a predator through recalling its scent or location at a particular time of day. For these reasons, the use of animal models to study episodic memory is justified.

A range of animal studies have shown that animals can exhibit different components of episodic memory, including recognition of objects (what), temporal order (when) memory and spatial memory (where) (Dere, et al., 2006). Object recognition is an important facet of episodic memory, but it is necessary to assess this ‘what’ component in conjunction with the ‘when’ and/or ‘where’ component. Aggleton and Brown (1999) suggest that recognition alone is processed by the perirhinal cortex, and not the hippocampal formation. Therefore tests that focus on recognition in isolation do not assess

episodic memory. Some examples of tests that assess the combined 'what, where and when' features of episodic memory include novelty-preference paradigms, one-trial object-place recognition, one-trial object-context recognition, Barnes maze, Morris water maze, radial arm maze, T-maze, and the star maze.

Eacott and colleagues (Eacott & Easton, 2007; Eacott, Easton, & Zinkivskay, 2005) used an E-maze to simultaneously test memory recall of object recognition (what), place recognition (where) and context recognition through manipulations of the context, objects and their location. Rats were exposed to the same E-maze apparatus three times. In the first exposure, rats experienced two objects within a particular context. Next, rats were exposed to the same two objects in opposite positions within a different context. The third phase was a test phase and involved exposure to one of the previous contexts with two copies of a single previous object. The object was now located in a place not previously associated with that context for that object (see Figure 1.1). During the testing phase, rats should exhibit higher exploration behaviour in the novel object-context location as it relies on a rats' innate preference for novelty. It also simultaneously tests memory recall of what was previously displayed, where in the maze it had previously been located and which context this information had been learned. This test has been shown to be sensitive to fornix lesions (Eacott, et al., 2005), which are directly linked to the extended hippocampal system (Aggleton, 2008; Aggleton & Brown, 1999) (this will be discussed further in Section 1.3). Lesions of the fornix have also been shown to be impaired in other spatial memory tests, including the Morris water maze (de Bruin, Moita, de Brabander, & Joosten, 2001; Eichenbaum, Stewart, & Morris, 1990), T-maze, radial-arm maze (Warburton, Baird, Morgan, Muir, & Aggleton, 2000) and Barnes maze (Whishaw, Hines, & Wallace, 2001).

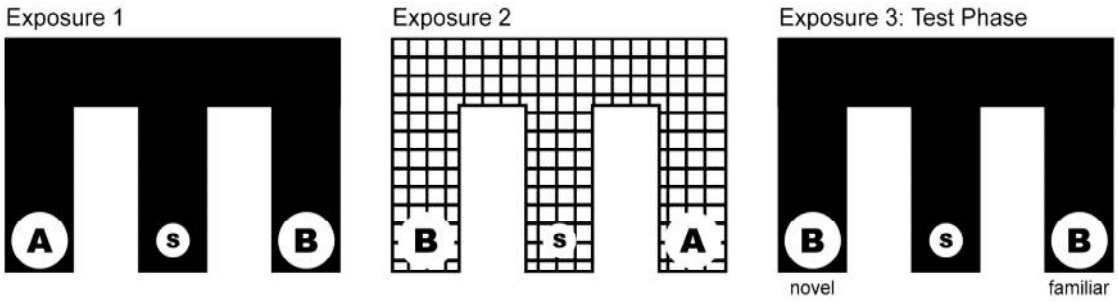

Figure 1.1. Outline of E-maze apparatus. Exposure 1: Black context present, objects not visible from start arm (S). Exposure 2: Mesh context present, objects not visible from start arm, and in positions opposite to those in the Black context. Exposure 3, Test phase: Black context with object B in a novel place relative to the context and a familiar place relative to the context. The test phase should result in higher exploration behaviour in the novel object-context location. Adapted from Eacott, et al., (2005).

A number of other studies have also examined the 'what' and 'where' features of episodic memory, but the temporal feature is often excluded (Baldi, Lorenzini, & Corrado, 2003; Benhamou & Poucet, 1998; Faraji, Lehmann, Metz, & Sutherland, 2008). Tulving (as cited in Fouquet, et al., 2010) emphasised that "the organization of knowledge in the episodic system is temporal. One event precedes, co-occurs, or succeeds another in time". An example of temporal order memory in rats (what and when) was carried out by Wolff and colleagues (2006). Rats were presented with a series of six randomly selected odours during the sample phase. During the single-choice test phase, recall of the temporal presentation of odours was tested, with the odour that had been presented earlier in the sequence rewarded. Rats with damage to the anterior thalamic nuclei exhibited impaired performance compared to control rats, showing that this region is involved in processing temporal information. As with the fornix, the anterior thalamic nuclei have been linked to the extended hippocampal system (Aggleton, 2008; Aggleton & Brown, 1999).

It is possible to test the three components of episodic memory (what, where and when) using food-storing birds (Clayton & Dickinson, 1998). Intact scrub jays were trained to cache either perishable worms (preferred food source) or non-perishable peanuts in visuospatially distinct locations. Jays searched preferentially for the perishable worms when the recall delay interval was short (4 hours), but modified their search to the non-perishable nuts after a longer interval (124 hours), during which the worms would have

perished. The retrieval preference demonstrated that scrub jays were able to recall what type of food had been cached where, and when the caching had occurred so as not to retrieve perished food. Although it is possible to test the three components of episodic memory in birds, the logistics of testing a variety of spatial memory tasks using ornithological lesion studies would be difficult and challenging. For this reason, it is more practical to use rodents for these types of tests.

Because testing rodents is relatively straightforward, there have been attempts to design comprehensive studies that include and test all three components of episodic memory. Rondi-Reig and colleagues (2006) created a paradigm that they claimed could test all three components of episodic memory (what, where and when) and could be assessed using both rodent and human subjects. They used a star-maze which comprised a total of ten alleys; five formed a central pentagon and five radiated from the angles of the central pentagon (see Figure 1.2). Subjects had to learn to find a hidden reward location from a single alley using either allocentric, sequential egocentric or serial strategies. They suggested that information regarding episodic memory came from the use of the sequential egocentric strategy. Subsequently, probe trials from a novel departure point tested whether subjects were able to use an allocentric navigation strategy, which would have resulted from learning the association between the goal location and the distal room cues (spatial information), an egocentric navigation strategy, which would have resulted from only learning the correct sequence, or a serial navigation strategy, in which subjects entered each alley and happened upon the goal location. Mice lacking NR1 receptors in the CA1 hippocampal field (NR1-KO) exhibited impairments across both allocentric and egocentric strategies, whereas control mice were able to use both navigation strategies. In human subjects, the star-maze task was carried out using virtual memory and performance was sensitive to age-related memory deficits (Fouquet, et al., 2010).

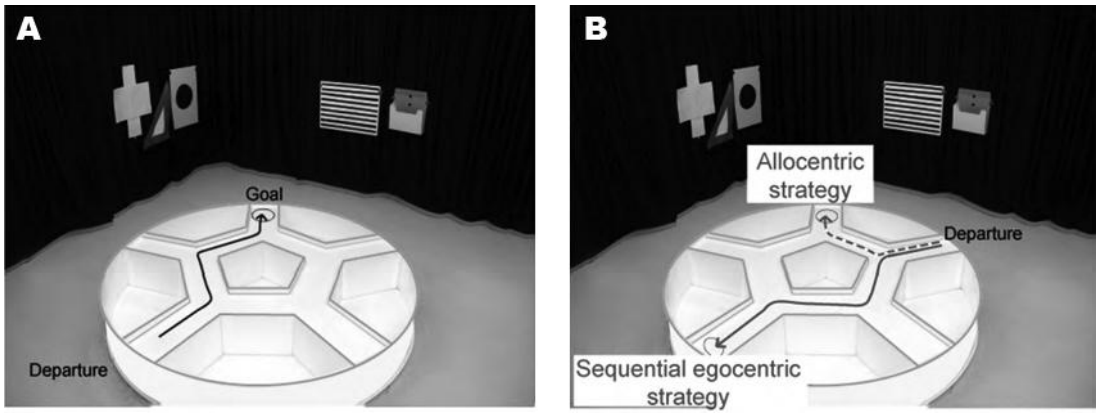

Figure 1.2. Illustration of the star-maze test during training and probe phases. During training, the departure and goal locations are fixed (A). Subjects are required to locate the goal location using either allocentric (spatial) or sequential egocentric (body turn) strategies. During the probe trial the departure location is shifted, but the goal remains fixed (B). This allows the two strategies to be identified within a single test. The use of spatial cues to locate the platform is shown as a dotted line whereas the use of the egocentric sequence (left-right-left) is shown as a solid line. Adapted from Fouquet, et al., (2010).

The task specifically involved learning a sequence of body turns in the correct order, for example left at the first intersection, right at the second intersection and left at the third intersection (Figure 1.2A). Rondi-Reig, et al., (2006) suggest that the ability to undertake this task demonstrated the retrieval of 'what' was required in the task (turn left or right), 'where' the turns had to occur (at the intersections) and 'when' the turn had to occur (according to the order in the sequence). However, while the authors claim this fulfils the requirements for episodic memory, the test was not designed to allow temporal order manipulations. Instead, the probes demonstrated 'what' was involved in the task (locate the hidden reward) and 'where' the hidden reward was located, but there was no specific temporal factor.

An alternative interpretation is that the task utilised procedural memory in conjunction with spatial memory. The rationale behind this interpretation is based on both H.M.'s ability to draw the floor plan after repeated exposures, as discussed earlier, and work of Cohen & Bacdayan (1994), who suggest that procedural memory relies on the repetition of specific procedures in order to perform a given task. For example, in the star-maze task, locating the hidden goal was learnt through repetition of the egocentric

sequence. Cohen & Bacdayan (1994) state that repetition of a given procedure typically results in improved performance over time, and that this is evident even when a subject is unable to declare that they have learnt the task. For example, dementia patients exposed daily to a given task, such as the Tower of Hanoi puzzle, are unable to recall the daily exposures, but show improvement on the task over time. As expected, in the star-maze task, through repeated exposures of the sequential egocentric procedure, performance improved over the twenty days of acquisition training. The sensitivity of the star-maze to hippocampal deficits in mice and declining cognitive ability in humans, implicates it as a useful spatial navigation memory test, but not a full episodic memory test.

In summary, tests of episodic memory can reliably be assessed using ornithological models, but these effects would be difficult to reproduce across different tests. In contrast, current rodent models only assess episodic-like memory by combining two of the three components. While it would be preferable to test all three facets of episodic memory using rodent models, a comprehensive test has not yet been designed to allow suitable manipulations of each component. In spite of this, evidence shows that combining two of the three episodic memory components still provides suitable information regarding episodic-like memory (what and where; what and when) using rodent models. As such, in the present study, rats will be required to find a chocolate food reward (what), located in a specific place relative to visual cues (where). Probes will be performed for four weeks, but manipulations of the temporal component will not occur.

1.2. Navigation

The ability of many animals to travel large distances, locate food and water sources, and return to their nests, demonstrates their ability to navigate using some form of mapping system (O'Keefe & Nadel, 1978). It has been postulated that accurate orientation and navigation involves the integrated use of location and directional heading (Stackman & Taube, 1997; Taube, 1995, 1998). It has been established that the hippocampus contains

"place cells", which specifically encode locations in space relative to the surrounding environment. These are reliant on the distance between the subject and landmarks, and are influenced by the proximity of the cues, i.e. proximal or distal (Burgess, 2008; Renaudineau, Poucet, & Save, 2007; Touretzky & Redish, 1996). It has also been established that the anterodorsal thalamic nucleus, one of the anterior thalamic nuclei, and laterodorsal thalamic nuclei contain head direction cells. Approximately 55 percent of the anterodorsal thalamic nuclei and 30 percent laterodorsal thalamic nuclei are direction sensitive (Mizumori & Williams, 1993; Taube, 1995). These cells fire when the head is aligned to a specific direction in space relative to one's body, but are not influenced by the specific location within an environment, nor olfactory, auditory or locomotor information (Mizumori & Williams, 1993). Similar to place cell firing, head direction cell firing relies heavily on the proximity of visual cues insomuch as the most prominent one exerts control over the firing pattern (Taube, 1998).

The firing of place cells in the hippocampus can be influenced by heading direction (Taube, 1998). Mizumori and colleagues (1994) postulated that hippocampal place cells integrate directional information from head direction cells which then facilitate navigation. Because of the connectivity between the hippocampus and the laterodorsal thalamic nuclei, they proposed that inactivation of head direction cells from the laterodorsal thalamic nuclei would disrupt the overall integrity of place cell firing in the hippocampus. In their study, rats were required to enter each arm of a radial arm maze once during a given trial. Error rates were recorded prior to, during, and post tetracaine injection, a local anaesthetic used to reversibly inactivate the laterodorsal thalamic nuclei. They found that when the laterodorsal thalamic nuclei were inactivated by tetracaine, the spatial distribution of firing patterns within the hippocampal place cells were altered and the number of errors increased. The change in firing patterns were not uniform, with some showing increased firing but decreased place specificity, some showing both reduced firing rates and

decreased place specificity, and others showing reduced firing rates but increased place specificity. The increased error rates observed in the radial arm maze task when the head direction cells were inactivated indicate that head direction cells do facilitate navigation, but it appears that other factors are also involved in navigation, as shown by the non-uniform firing patterns within the hippocampal place cells.

Wilton and colleagues (2001) looked at the effects of removing the anterodorsal and laterodorsal thalamic nuclei (AD+LD), two key structures that contain head direction cells, on two spatial memory tasks. The first was a T-maze non-matching to place alternation task and the second was a modified water maze task. In the first run of a trial on the T-maze task, rats were forced to enter one arm of the maze and then in the second they were rewarded with food if they chose the alternate arm. The modified water maze task involved searching for a hidden platform using either distal room cues to build a spatial map, or by using a heading trajectory based on beacon that was always placed at a fixed distance and direction from the platform. They found that lesions to the AD+LD resulted in consistently higher error rates on the T-maze alternation task and longer latencies in the water maze beacon task compared to the control group. These results implicate heading direction cells in accurate spatial navigation across two different tasks that have quite different characteristics. Along with heading direction, the T-maze implements a linear search strategy to a specific place insomuch as the rat can only follow a single path. On the other hand, the water maze makes allows a more general search strategy to a specific place that is not restricted to a single path.

The use of direction and place to guide navigation are thought to be reliant on salient cues or spatial points of reference. Hamilton and colleagues (2004) have labelled these as ‘cued navigation’ and ‘place navigation’ respectively. Cued navigation involves finding a target location using a single salient cue that directly marks the target, e.g. a beacon, and relies on egocentric navigation strategies. Egocentric navigation involves integration of

sensory and motor skills including proprioceptive, vestibular and somatosensory signals (Burgess, 2008). Conversely, place navigation involves finding a target location using a fixed arrangement of external cues, none of which specifically mark the target and relies on allocentric (spatial) navigation strategies (Hamilton, et al., 2004; O'Keefe & Nadel, 1978). A simple T-maze task can differentiate these two strategies (Figure 1.3) where the test phase uses a rotated maze and examines whether a rat can return to the food reward (matching to place) using allocentric navigation, or whether it turned right as per the training phase (cue) using egocentric navigation.

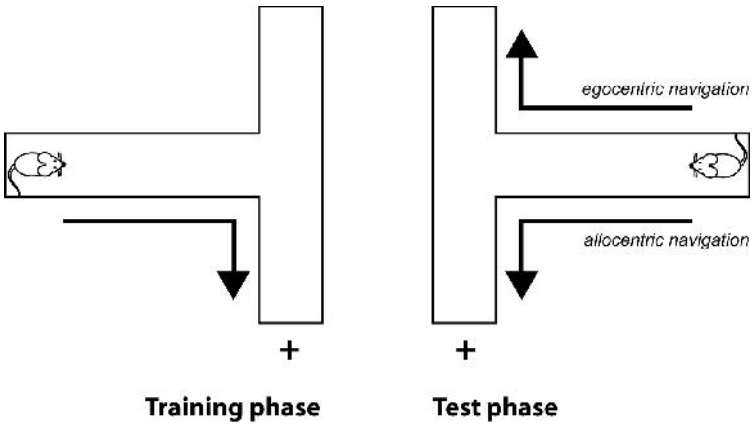

Figure 1.3. A schematic diagram showing a T-maze task to test egocentric and allocentric navigation strategies (not investigated in the present study). The rat would receive a food reward during training when it turned right. The maze would then be rotated and the rat tested again. A right turn indicated an egocentric strategy (cued), and a left turn indicated an allocentric strategy (place). Adapted from Eichenbaum, (2002).

O'Keefe and Nadal (1978) suggest that place and cued strategies are governed by different neural systems: the medial temporal lobe, and the parietal and occipital regions, respectively. Place navigation is dependent on the medial temporal lobe system and requires "that an animal construct, store and routinely update a cognitive map of the environment" (Hamilton, et al., 2004), thus creating a flexible representation of space (Aggleton, 2008; Aggleton & Brown, 1999; Eichenbaum, et al., 1990; Hamilton, et al., 2004; Renaudineau, et al., 2007; Rossier, Grobety, & Schenk, 2000; Save & Moghaddam, 1996; Whishaw & Tomie, 1997). Furthermore, as Aggleton and colleagues (Aggleton, 2008; Aggleton & Brown, 1999) have shown, the extended hippocampal system

incorporates regions of the diencephalon, which has also been implicated in place navigation.

Cued navigation, on the other hand, does not require the use of a cognitive map or the medial temporal lobe system, but rather involves an egocentric spatial system believed to depend on the parietal and occipital regions. These regions integrate visual, proprioceptive and vestibular information to monitor movement along a trajectory, often established by a single salient cue (Burgess, 2008; O'Keefe & Nadel, 1978; Save & Moghaddam, 1996). When both navigation strategies are used in parallel, Burgess (2008) suggests that reciprocal processing of allocentric and egocentric information is mediated by the retrosplenial cortex (Figure 1.4).

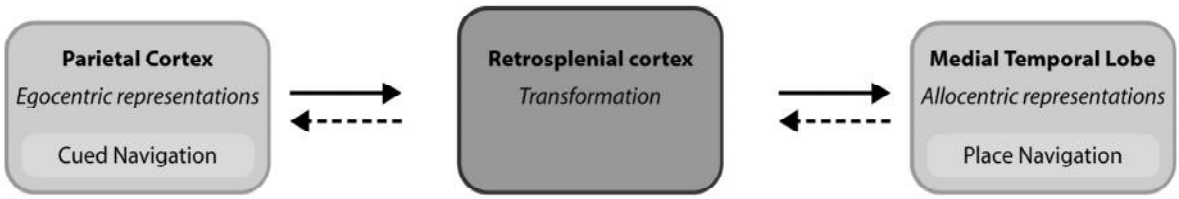

Figure 1.4. Schematic representation of reciprocal egocentric/allocentric information processing during navigation. The solid lines represent 'bottom-up' connections from egocentric to allocentric regions; dashed lines represent 'top-down' connections back again. Adapted from Burgess, (2008).

Incorporation of both place and cued navigation is likely to occur in natural settings where visual cues can be salient and/or part of a fixed arrangement. However, under certain circumstances animals may preferentially use one strategy over the other. Where movement involves long-distance navigation, it would be more efficient to form a spatial representation of the environment (cognitive map) and update one's position within that, rather than keeping track of each egocentric movement which may result in a high error rate. Conversely, short distances do not require the complexity of a cognitive map, and therefore implementation of an egocentric strategy would be more efficient (Burgess, 2008).

1.2.1. Effects of Cue Proximity on Spatial Navigation

As mentioned above, the firing of place cells and head direction cells, both involved in spatial navigation, can be influenced by the proximity of visual cues. Shapiro, et al., (1997) postulated that rats respond to visual cues in a hierarchical manner, with place cells encoding the relationship between proximal and distal cues first, distal cues in isolation next, and lastly, proximal cues in isolation. Thus, navigation using both proximal and distal cues would be more accurate than navigating with a single cue. Renaudineau, et al., (2007) also advocate a hierarchy theory, but suggest it should be regarded as a flexible system, as some place cells respond to both proximal and distal cues, while others predominantly respond to a single cue type. Nonetheless, in support of Shapiro's hierarchical theory (1997), both human (Cánovas, García, & Cimadevilla, 2011) and animal (Brett, 2008; Save & Poucet, 2000) studies show poorer performance when proximal cues guide navigation compared to when distal cues guide navigation across a number of spatial memory tests. For example, using a virtual memory 'box room' task, similar to the hole-board maze used for mice, Cánovas, et al., (2011) tested the effect of cue proximity on place navigation in human subjects. They found that subjects who used proximal cues to locate the reward place were slower and less accurate than subjects who used distal cues. Similar results were seen in two animal studies, using different variations of the water maze task: navigation reliant on proximal cues was poorer than when reliant on distal cues. These studies also highlighted the influence of the medial temporal lobe and diencephalic brain regions on spatial navigation compared to the parietal cortex (Brett, 2008; Save & Poucet, 2000).

1.3. Anatomical Considerations of the Extended Hippocampal System

As mentioned earlier, literature on both human and animals have shown that pathology in the medial temporal lobe (including the hippocampal formation) results in anterograde amnesia. Furthermore, the diencephalon, which comprises the thalamus and hypothalamus, has also been shown to be involved in the formation of episodic memories (Aggleton, 2008; Aggleton & Brown, 1999; Fleischman & Gabrieli, 1999). Because the clinical impairment after pathology to either region is similar, studies have focussed on the neural connectivity between them (Aggleton, 2008; Aggleton & Brown, 1999; Aggleton & Pearce, 2001; Fleischman & Gabrieli, 1999; Scoville & Milner, 2000; Squire, 1992). Together, specific structures from the diencephalon and medial temporal lobe regions, including the hippocampus, fornix, anterior thalamus and the mammillary bodies, form the "extended hippocampal formation" (Aggleton & Brown, 1999). Thus far, it is unclear whether the laterodorsal thalamic nuclei contribute to, and function as part of the extended hippocampal formation.

The laterodorsal thalamic nuclei were previously categorised as part of the anterior thalamic aggregate, i.e. lateral nucleus pars anterior (van Groen & Wyss, 1992). However, through structural and connectivity studies, it has been shown that the anterior and laterodorsal thalamic nuclei are separate, adjacent structures (see Figure 1.5) which receive similar neural connections from the hippocampal formation (Aggleton, 2008; Shibata & Naito, 2005; van Groen & Wyss, 1992). As they are separate structures, it is appropriate to consider the distinct differences in neural connectivity. As the focus of this thesis is on the contribution of the anterior and laterodorsal thalamic nuclei on spatial learning and memory, these two structures will be discussed in more detail in Sections 0 and 1.3.2.

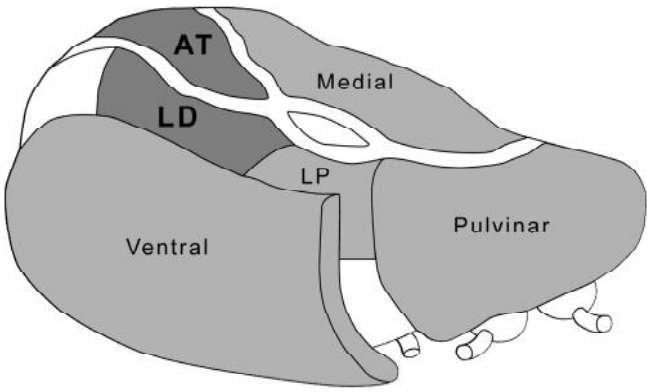

Figure 1.5. Diagrammatic representation of the thalamus. Abbreviations: AT = anterior thalamic nuclei; LD = laterodorsal thalamic nuclei; LP = lateroposterior thalamic nuclei. Adapted from Austin (2003).

Studies by Aggleton and colleagues (Aggleton, 2008; Aggleton & Brown, 1999; Aggleton, Neave, Nagle, & Hunt, 1995; Aggleton & Pearce, 2001; Warburton & Aggleton, 1999; Warburton, et al., 2000) have shown that formation of episodic memories relies on the effective function of each component of the neural circuit that makes up the extended hippocampal system (Figure 1.6), and damage to individual components can produce deficits across a range of spatial reference memory tasks. For example, in the standard Morris water maze task, in which rats have to search for a hidden platform, sham-lesioned rats show reduced escape latencies and path lengths over time, indicative of learning and memory. However, rats with a lesion to the hippocampus, fornix or anterior thalamus show longer escape latencies and path lengths, indicating disrupted learning. This suggests that, initially, lesioned rats are not able to use efficient and effective search strategies to find the hidden platform, and, secondly, recall of the platform location is diminished. The same pattern of impaired spatial learning after these lesions has been shown in the radial-arm maze and the T-maze.

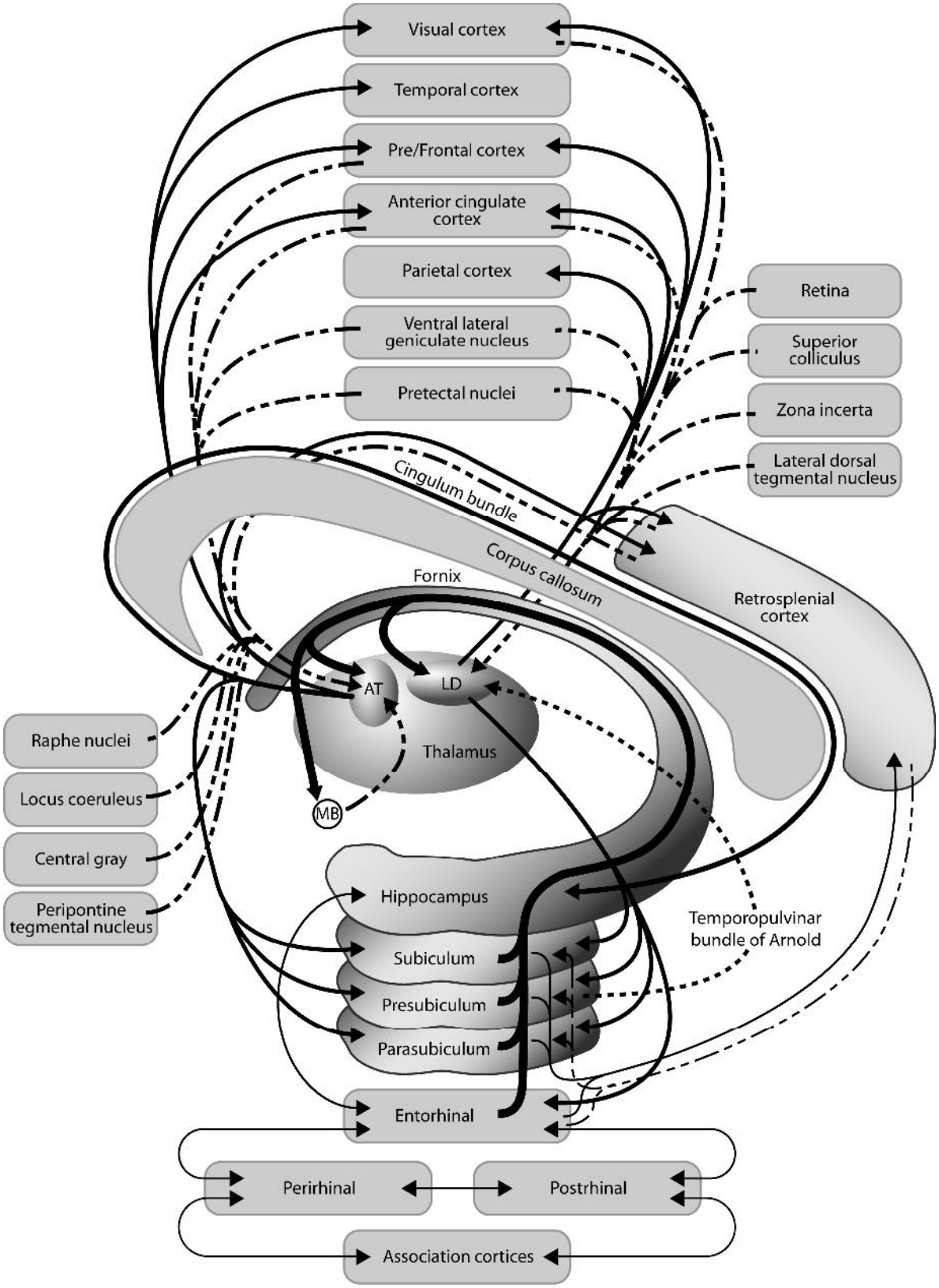

Figure 1.6. Diagrammatic representation of the neural connections of the extended hippocampal formation and the contrasting sets of connections between the anterior thalamic nuclei and laterodorsal thalamic nuclei. The thick solid lines represent the connections between the hippocampus and the two thalamic nuclei. The thin solid lines represent the efferent connections. The dotted lines represent the afferent connections. Abbreviations: AT = anterior thalamic nuclei; LD = laterodorsal thalamic nuclei; MB = mammillary bodies.

Figure 1.6 illustrates the extended hippocampal formation and the contrasting sets of connections for the anterior thalamic nuclei and laterodorsal thalamic nuclei that support learning and memory. The critical components involved in processing episodic memory include dense efferent projections from the hippocampal formation to the thalamus and mammillary bodies, and these originate in the subicular complex and the entorhinal complex and pass, primarily, via the fornix (Aggleton, 2008). Studies have shown that damage to the fornix can permanently impair learning and memory, with a disproportionate loss of episodic memory (Aggleton et al., 2010; de Bruin, et al., 2001; Warburton, et al., 2000; Whishaw, et al., 2001).

Direct reciprocal projections from the thalamus to the hippocampal formation pass through the cingulum bundle, a white matter tract that facilitates the transfer of information. Damage to the cingulum bundle interrupts information transmission within the extended hippocampal formation. Damage to this fibre tract impairs episodic memory, but to lesser extent than damage to the hippocampus, anterior thalamic nuclei or mammillary bodies (Aggleton & Brown, 1999; Warburton, Aggleton, & Muir, 1998). The neural connections beyond these key structures within the extended hippocampal system become more diffuse, but are nonetheless important in learning and memory (Aggleton & Brown, 1999).

1.3.1. Anterior Thalamic Nuclei

The anterior thalamic structure is made up of three main aggregates: the anterodorsal thalamic nucleus (AD), the anteroventral thalamic nucleus (AV) and the anteromedial thalamic nucleus (AM). Through selective lesions studies, each of these aggregates has been shown to be moderately involved in spatial learning and memory (van Groen, Kadish, & Wyss, 2002a). However, when all three aggregates were destroyed, the deficits seen were much greater. For this reason, anterior thalamic lesions in the current study were targeted at the whole structure. This section will focus on the specific neural connectivity

of the anterior thalamic nuclei within the framework of the extended hippocampal formation (Figure 1.6).

Clinical evidence of diencephalic amnesia in patients with Korsakoff's syndrome is typically associated with atrophy of the mammillary bodies and the anterior thalamic nuclei (Gold & Squire, 2006; Kopelman, Thomson, Guerrini, & Marshall, 2009). These two structures receive direct projections from the hippocampal formation. The mammillary bodies have efferent projections solely to the anterior thalamus (Aggleton, 2008; Aggleton & Brown, 1999), while the anterior thalamic nuclei project back to the hippocampal formation directly, via the cingulum bundle (Aggleton & Brown, 1999). The efferent projections from the mammillothalamic tract to the anterior thalamus have been shown to be involved in visuospatial memory, an element of episodic memory, implicating it as a structure of the extended hippocampal system (Vann, Honey, & Aggleton, 2003; Vann, Saunders, & Aggleton, 2007).

The anterior thalamic nuclei and hippocampal formation also have reciprocal connections with the retrosplenial cortex (Vann, Aggleton, & Maguire, 2009). Aggleton and colleagues (Aggleton, 2008; Aggleton, et al., 2010; Aggleton & Pearce, 2001; Vann, et al., 2009) have shown that damage to the hippocampus, anterior thalamic nuclei or the mammillary bodies results in covert pathology in the retrosplenial cortex, also implicating it in episodic memory processing and as a critical component of the extended hippocampal formation. Reciprocal connections are also found between the anterior thalamic nuclei and the pre-frontal/frontal cortex, a region implicated in the processing of executive functions including the integration of temporally separate events, memory and recall (Aggleton, 2008; Aggleton & Brown, 1999; Aggleton, et al., 2010; Aggleton & Pearce, 2001; Lezak, Howieson, & Loring, 2004), and the anterior cingulate cortex, a region implicated in response selection and attention (Aggleton, et al., 2010; Horikawa, Kinjo, Stanley, & Powell, 1988; Lezak, et al., 2004).

In addition to the critical connections of the extended hippocampal formation, more diffuse afferent anterior thalamic nuclei projections emerge from the ventral lateral geniculate nucleus, pretectal nuclei, raphe nuclei, locus coeruleus, central gray and the peripontine tegmental nucleus (Sikes & Vogt, 1987). Many of these subcortical regions allow auditory and visual information to be relayed to the anterior thalamic nuclei. The anterior thalamic nuclei also play a role in the distribution of information, as seen by the efferent projections to the visual cortex (van Groen, Kadish, & Wyss, 1999) and the temporal cortex (Aggleton & Brown, 1999).

1.3.2. Laterodorsal Thalamic Nuclei

The laterodorsal thalamic structure is made up of two main aggregates: the dorsomedial thalamic nucleus (LDDM) and the ventrolateral thalamic nucleus (LDVL). It sits adjacent and posterior to the anterior thalamic nuclei, and has been implicated in spatial learning and memory (Aggleton, 2008; van Groen, Kadish, & Wyss, 2002b). This section will focus on the specific neural connectivity of the laterodorsal thalamic nuclei within the framework of the extended hippocampal formation (Figure 1.6).

In contrast to the anterior thalamic nuclei, the laterodorsal thalamic nuclei have two parallel afferent projections from the hippocampal formation (Aggleton, 2008; Aggleton & Brown, 1999). The first projects via the fornix and the second projects from the presubiculum via the temporopulvinar bundle of Arnold (Aggleton, 2008). However, unlike the anterior thalamic nuclei, the laterodorsal thalamic nuclei do not project to, nor do they receive projections from the mammillary bodies (Vann, et al., 2007). The laterodorsal thalamic nuclei also project directly back to the hippocampal formation rather than via the cingulum bundle as with the anterior thalamic nuclei.

In addition to the reciprocal projections with the hippocampal formation, there is also evidence of reciprocal projections with the retrosplenial cortex, which has been implicated in spatial memory (Aggleton, 2008; Shibata, 2000; Shinkai et al., 2005), the anterior

cingulate cortex which has been implicated in response selection and attention (Lezak, et al., 2004; Shibata & Naito, 2005), and the visual cortex, which processes visual information (Shinkai, et al., 2005; van Groen & Wyss, 1992). In contrast to the anterior thalamic nuclei, the laterodorsal thalamic nuclei have solely efferent projections to the prefrontal cortex (Aggleton & Brown, 1999), and do not project to the temporal cortex. Unlike the anterior thalamic nuclei, they do, however, project to the parietal cortex (Chandler, King, Corwin, & Reep, 1992; Kolb & Walkey, 1987; Reep, Chandler, King, & Corwin, 1994), which has been implicated in egocentric navigation (Burgess, 2008).

Outside of the extended hippocampal formation, more diffuse afferent connections to the laterodorsal thalamic nuclei originate in the ventral lateral geniculate nucleus, pretectal nuclei, retina, superior colliculus (Shinkai, et al., 2005), lateral dorsal tegmental nucleus and the zona incerta (Ryszka & Heger, 1979). Many of these non-visual connections indicate that the laterodorsal thalamic nuclei are involved in multisensory information. Furthermore, sequential connections indicate that these nuclei are also involved in processing visual information (Shinkai, et al., 2005). For example, the hippocampal formation receives visual information via the retina→superior colliculus→laterodorsal thalamic nuclei→subicular complex circuit (Mizumori & Williams, 1993).

1.4. Summary of Anterior and Laterodorsal Thalamic Lesion Studies

In conjunction with neural connectivity studies, a number of behavioural studies have been performed to ascertain the effects of anterior and laterodorsal thalamic lesions on spatial memory processing and their contribution to the extended hippocampal system. Table 1 provides an overview of studies undertaken on rats that include lesions to the anterior thalamic nuclei and/or the laterodorsal thalamic nuclei. From this, it is evident that lesions to the anterior thalamic nuclei, or its individual aggregates, consistently produce spatial memory deficits across a number of spatial memory tasks, thus strongly implicating it as a major contributor to the extended hippocampal system. However, across the same tasks,

the smaller number of studies with lesions to the laterodorsal thalamic nuclei has produced inconsistent results. As discussed below, these inconsistencies may be attributable to the varied nature and demands of the tasks.

Two studies indicated that lesions restricted to the laterodorsal thalamic nuclei produced mild impairments on a spatial 'working' memory task, in which rats had to acquire and retain a new spatial location on a daily basis (Brett, 2008; van Groen, et al., 2002b). When tested on spatial 'reference' memory, one study indicated that rats with selective laterodorsal thalamic nuclei lesions did not produce any impairments (Craw, Rapley, Wolff, Kesner, & Dalrymple-Alford, 2007). However, when using the same experimental procedures as Craw, et al., (2007), Brett (2008) found that rats with laterodorsal thalamic nuclei lesions were mildly impaired across acquisition, but showed no impairment in the probe trials. In addition to the differing memory demands of these tasks, the discrepancy between these studies could be due to lesion size and location, but histology analysis awaits completion (Brett, 2008; Craw, et al., 2007).

Table 1. Summary of studies on spatial memory tasks after lesions of the anterior and/or laterodorsal thalamic nuclei.

Year	Authors	Lesion Site(s)	Lesion Method	Behavioural Tasks	Deficits
2011	Aggleton, Amin, Jenkins, Pearce, & Robinson	AT	NMDA	1. Sequence learning 2. T-maze - spatial alternation	AT Impaired on #2
2010	Dumont, Petrides, & Sziklas	FX+RSP AT+HPC+RSP ipsi AT+HPC+RSP contra	Ibotenic acid Electrolytic	1. Visuospatial conditional associative task 2. 8-arm radial maze	FX+RSP Impaired on #1 and #2 AT+HPC+RSP *i* Impaired on #1 and #2 AT+HPC+RSP *c* Impaired on #1 and #2
2009	Lopez, Wolff, Lecourtier, Cosquer, Bontempi, Dalrymple-Alford & Cassel	AT ILN+LT	NMDA	1. Morris water maze acquisition with delayed re-testing (5 days and 25 days)	AT Impaired at 5d and 25d delay ILN+LT Impaired at 25d delay only
2008*	Brett	AT LD	NMDA	1. Morris water maze (proximal cues) 2. Morris water maze (distal cues) 3. Morris water maze (working memory)	AT Impaired on #1, #2 and #3 LD Minimally impaired on #2 and #3
2008	Wolff, Gibb, Cassel & Dalrymple-Alford	AT ILN	NMDA	1. Morris water maze 2. 8-arm radial water maze (egocentric)	AT Impaired on #1 ILN Not impaired on #1 or #2
2007*	Craw, Rapley, Wolff, Kesner & Dalrymple-Alford	AT LD	NMDA	1. 12-arm radial maze 2. Morris water maze	AT Impaired on #1 (transient) and #2 LD Not impaired on #1 or #2
2007	Sziklas & Petrides	AT	Electrolytic	1. Visuospatial conditional associative task 2. 8-arm radial maze	AT Impaired on #2
2006	Gibb, Wolff & Dalrymple-Alford	AT LT MT	NMDA	1. Odour-place paired-associate task 2. Odour and spatial discrimination task	AT Impaired on #1 LT Impaired on #1 MT Not impaired on 1# or #2
2006	Mitchell & Dalrymple-Alford	AT LT	NMDA	1. Plus maze 2. 8-arm radial maze	AT Impaired on #2 LT Impaired on #1
2005	Mitchell & Dalrymple-Alford	AT LT MT	NMDA	1. 12-arm radial maze 2. Go-no go reward magnitude 3. Temporal order memory 4. Familiar vs. novel object recognition	AT Impaired on #1 LT Impaired on #3 MT Impaired on #2 and #3

Year	Authors	Lesion Site(s)	Lesion Method	Behavioural Tasks	Deficits	
2004	Henry, Petrides, St-Laurent & Sziklas	AT*x*HPC contra	Ibotenic acid	1. Visuospatial conditional associative task 2. Delayed forced alternation	AT*x*HPC	Impaired on #1 and #2
2003	Mair, Burk & Porter	AT PH AT+PH	NMDA RF	1. 8-arm radial maze	AT PH AT+PH	Impaired on #1 Impaired on #1 Impaired on #1
2003	Moran & Dalrymple-Alford	AT PRC	NMDA	1. 12-arm radial maze 2. Spatial configuration task 3. Spontaneous object recognition task	AT PRC	Impaired on #1 Impaired on #2
2002	van Groen, Kadish & Wyss	AD+AV AD+AV+ AD+AV+AM	Ibotenic acid	1. Morris water maze	AD+AV AD+AV+ AD+AV+AM	Impaired on #1 Impaired on #1 Impaired on #1 – most severe
2002*	van Groen, Kadish & Wyss	LD LD+AD+AV	Ibotenic acid	1. Morris water maze	LD LD+AD+AV	Minimally impaired on #1 Impaired on #1 – most severe
2001	Warburton, Baird, Morgan, Muir & Aggleton	AT+HPC ipsi AT+HPC contra	NMDA	1. T-maze alternation task 2. 8-arm radial maze 3. Morris water maze	AT+HPC *i* AT+HPC *c*	Minimally impaired on #1, #2 or #3 Impaired on #1, #2 and #3
2001*	Wilton, Baird, Muir, Honey & Aggleton	AD+LD	NMDA	1. T-maze alternation task 2. Morris water maze 3. Object-in-place task 4. Spontaneous object recognition task	AD+LD	Impaired on #1, #2 and #3
2000	Warburton, Baird, Morgan, Muir & Aggleton	AT+FX ipsi AT+FX contra FX AT+FX contra+HPC	NMDA RF	1. Object recognition task 2. Object location task 3. T-maze alternation task 4. Morris water maze 5. 8-arm radial maze 6. T-maze alternation task	AT+FX *i* AT+FX *c* FX AT+FX*c*+HPC	Impaired on #2, #3, #4, #5 and #6 Impaired on #2, #3, #4, #5 and #6 Impaired on #2, #3, #4, #5 and #6 Impaired on #2, #3, #4, #5 and #6 - most severe
1999	Sziklas & Petrides	AT	Electrolytic	1. 8-arm radial maze 2. Visuospatial conditional associative task 3. T-maze (egocentric)	AT	Impaired on #1 and #2

Year	Authors	Lesion Site(s)	Lesion Method	Behavioural Tasks	Deficits
1999	Warburton & Aggleton	AT FX	NMDA RF	1. Morris water maze 2. T-maze alternation task 3. Spontaneous object recognition task	AT Impaired on #1 and #2 FX Impaired on #1 and #2
1999	Warburton, Morgan, Baird, Muir & Aggleton	AT FX	NMDA RF	1. Morris water maze 2. T-maze alternation task	AT Impaired on #1 and #2 FX Impaired on #1 and #2
1997*	Warburton, Baird & Aggleton	AT AT+LD FX	NMDA RF	1. T-maze alternation task 2. Cross maze (allocentric) 3. Cross maze (egocentric)	AT Impaired on #1 and #2 AT+LD Impaired on #1 and #2 FX Impaired on #1 and #2
1996	Aggleton, Hunt, Nagle & Neave	AM AV+AD AT	NMDA	1. T-maze alternation task 2. Egocentric discrimination task 3. 8-arm radial maze	AM Impaired on #1 AV+AD Impaired on #1 and #3 AT Impaired on #1 and #3 – most severe
1996	Byatt & Dalrymple-Alford	AM AV	RF	1. 12-arm radial maze working memory 2. 12-arm radial maze reference memory	AM Impaired on #1 and #2 AV Impaired on #1 and #2
1995	Aggleton, Neave, Nagle & Hunt	AT MB FX	NMDA RF	1. T-maze alternation task 2. Spontaneous object recognition	AT Impaired on #1 MB Impaired on #1 FX Impaired on #1

Abbreviations: AD = anterodorsal thalamic nucleus; AM = anteromedial thalamic nucleus; AT = anterior thalamic nuclei; AV = anteroventral thalamic nuclei; Contra = contralateral; FX = fornix; HPC = hippocampus; ILN = intralaminar thalamic nuclei; Ipsi = ipsilateral; LD = laterodorsal thalamic nuclei; LT = lateral thalamic nuclei; MB = mammillary bodies; MT = medial thalamic nuclei; NMDA = *n*-methyl-D-aspartate; PH = parahippocampal cortex; PRC = perirhinal cortex; RF = radio frequency; RSP = retrosplenial cortex; * indicates studies that specifically include the laterodorsal thalamic nuclei.

Further evidence implicating the laterodorsal thalamic nuclei in the processing of spatial memory comes from two studies which examined the effect of combined lesions to both the anterior and laterodorsal thalamic nuclei (AT+LD) (van Groen, et al., 2002b; Warburton, et al., 1997). Consistently poorer performance was observed in the combined lesion group compared to control rats and anterior thalamic (AT) or fornix lesions (FX) across all spatial memory tasks. For example, compared to the LD lesion group, the combined AT+LD lesion group had longer latency to locate the hidden platform, and showed minimal improvement over the five days of testing in a spatial working memory task (van Groen, et al., 2002b). On an allocentric alternation task (cross maze), in which the rat was rewarded if it returned to the food well from the opposite start point, the combined AT+LD lesion group showed much higher error rates compared to the FX lesion group (Warburton, et al., 1997). In addition to the spatial memory tasks, these studies often tested egocentric memory and/or object recognition. The most notable difference was that the lesion groups now showed no impairments.

1.5. Aims of the Present Study

The previous summary established that lesions to the anterior thalamic nuclei produce deficits in spatial memory tasks, but the outcome of selective lesions to the laterodorsal thalamic nuclei produce inconsistent results with few studies conducted. Thus, the primary aim of this study was to compare spatial reference memory in rats with selective lesions to either the anterior thalamic nuclei or the laterodorsal thalamic nuclei. The secondary aim was to assess the influence of cue proximity on the propensity to use egocentric or allocentric navigation strategies when locating a specific place and to determine whether navigation strategies altered over time. These objectives were accomplished in a single study.

Rats were required to learn the location of a food reward using a spatial configuration and/or a fixed trajectory. The spatial configuration comprised proximal or

distal visual cues, a beacon indicating the location of the food reward and a fixed start point relative to the visual cues and beacon. The fixed trajectory was established through the fixed relationship between the start point and beacon location. This setup provided rats the opportunity to establish different navigation strategies during training, i.e. egocentric and allocentric. Strategy performance was then probed weekly for four weeks by 1) removing the beacon only to test general navigation, 2) removing the beacon and starting from a novel start point relative to the standard configuration to test allocentric navigation or 3) removing all visual cues to test egocentric navigation.

As suggested in the current literature (Table 1), it was expected that rats with lesions to the anterior thalamic nuclei would produce clear deficits in tasks that test allocentric navigation, but rats with laterodorsal thalamic lesions would exhibit mild or no impairments. In comparison, when tested on egocentric navigation tasks, rats with anterior thalamic lesions were expected to be unimpaired. As shown in Table 1, there is no current literature on the performance of rats with lesions restricted to the laterodorsal thalamic nuclei in egocentric tasks, but because of the neural connections with the parietal cortex, a structure implicated in egocentric processing, it was hypothesised that performance would be impaired in rats with lesions to laterodorsal thalamic nuclei when egocentric strategies were required for navigation. Furthermore, in support of the current but small human and animal literature, it was expected that this study would also elicit poorer performance across both deviation scores and latency measures when proximal cues guided navigation compared to when distal cues guided navigation (Brett, 2008; Cánovas, et al., 2011; Save & Poucet, 2000).

In summary, it was expected that, overall, performance would be poorer in the proximal cue condition compared to the distal cue condition. Additionally, the allocentric probe in this task would elicit deficits in rats with anterior thalamic lesions and produce mild to no deficits in rats with laterodorsal thalamic lesions. In contrast, the egocentric

probe in this task would elicit no deficits in rats with anterior thalamic lesion and produce deficits in rats with laterodorsal thalamic lesions. It was also expected that performance would improve over the four week probe regime as rats learnt the task.

2. Methods

2.1. Subjects

Fifty-four female PVGc hooded rats bred in the Psychology department at the University of Canterbury were used. They were housed in groups of three to four in standard opaque plastic cages (27 cm wide x 45 cm long x 22 cm high). The colony room was maintained at 22˚C and 48% rH under a reversed 12 hour light schedule (off 0800 h to 2000 h). Behavioural testing was conducted during the dark phase of the cycle. Rats were maintained around 85% - 90% free feeding weight and water was available ad libitum during testing. At surgery, rats were approximately 12 months old and weighed between 155 g and 220 g. During post-surgery recovery all rats were housed individually. Prior to testing all rats were re-housed into groups of three or four. All protocols conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of the University of Canterbury.

2.2. Surgery

Prior to surgery, rats were randomly assigned to one of three groups: anterior thalamic lesions (AT: *n = 18*), laterodorsal thalamic lesions (LD: *n = 18*) or sham-operated (SHAM: *n = 18*). All surgical procedures were carried out under aseptic conditions. Rats were anaesthetised intraperitoneally (IP) using ketamine and Domitor (medetomidine) (for doses, see Table 2.1) and then administered Hartmann's solution.

Table 2.1. Drug doses used during surgery.

Drug	Concentration	Dose (solution)	Dose Volume (surgery)
Ketamine	100 mg/mL	75 mg/mL	1 mL/kg
Domitor (medetomidine)	1 mg/mL	0.35 mg/mL	1 mL/kg
Antisedan (atipamezole)	5 mg/mL	1.75 mg/mL	1 mL/kg
Norocarp (carprofen)	50 mg/mL	25 mg/mL	0.1 mL
Mepivacaine	20 mg/mL	2 mg/mL	0.2 mL

1mL of Hartmann's solution was administered at the start and another 1 mL at the end of surgery.

The incisor bar of the stereotaxic (Kopf, Tujunga, CA) was set at 7.5 mm below the interaural line to minimise fornix injury. Norocarp (carprofen), an anti-inflammatory, was injected into the nape of the neck. An incision was made in the scalp, and the skin retracted to expose the skull. Mepivacaine, a local anaesthetic, was administered on the exposed skull to diffuse through the skin and cranial membranes. A craniotomy was made above the target coordinates, outlined in Table 2.2, and the dura was cut.

Table 2.2. Methodology for AT and LD lesions: coordinates (cm) for various Bregma-Lambda (B-L) measurements, infusion volumes and rates using 0.15 M NMDA.

	Corresponding AT coordinates			LD coordinates
B-L distance for coordinates (cm)	**Anterior (AM)**	**Posterior (AV)**		**Single Site**
≤0.62	-0.230	-0.240		
0.63 – 0.65	-0.235	-0.245		
0.66 – 0.68	-0.240	-0.250		
≥0.69	-0.245	-0.255		
<0.63				-0.330
0.64 – 0.66				-0.335
≥0.67				-0.340
M-L Distance	±0.118	±0.152		±0.222
D-V Distance	-0.583	-0.555	-0.565	-0.482
NMDA Volume (μL)	0.18	0.12	0.18	0.20
Infusion Rate (μL/min)	0.03	0.03	0.03	0.03

Abbreviations: AM = anteromedial; AT = anterior thalamic nuclei; AV = anteroventral; B-L = bregma to Lambda; D-V = dorsal to ventral; LD = laterodorsal thalamic nuclei; M-L = medial to lateral

Lesions were made using micro-infusions of 0.15 M N-methyl-D-aspartate (NMDA; Sigma Chemicals, Australia) dissolved in phosphate buffer, pH 7.20, via a 1 μL Hamilton syringe (needle: 25S, outer diameter 0.51 mm; inner diameter 0.13 mm) connected to a motorised micro-infusion pump (Stoelting, Reno, USA) and remained in situ for 3 minutes post-infusion for diffusion (for details, see Table 2.2). The sham procedures were identical to that just described, except that the Hamilton syringe was lowered to 1.5 mm above the lesion coordinates and no material was infused. After the skin was sutured, Emla cream

(topical anaesthetic) was applied and the sedative reversal drug Antisedan (atipamezole) and Hartmann's solution administered. Rats were ear marked for identification.

2.3. Apparatus

2.3.1. Cheeseboard Maze

The white circular cheeseboard maze, located in a windowless room (3.4m x 3.4m), was 1500 mm diameter, 40 mm thick, and raised 740 mm off the floor. The cheeseboard was constructed from two 18 mm thick pieces of wood with wire mesh between the two. The upper board had 223 food wells (25 mm diameter x 15 mm deep). The lower board of the apparatus was 1200 mm in diameter with 177 wells (25 mm diameter x 15 mm deep), each partially filled with 0.1 g of chocolate chips (Nestlé Chocettes™ Dark Compound, New Zealand). These were inaccessible from the upper board, and present in order to control for food odour cues.

2.3.1.1. Proximal Cue Rotation System

A 2500 mm diameter beige curtain surrounded the cheeseboard, and sheets of black paper were fastened to the ceiling to minimise overhead external cues (i.e. camera cables). A 1500 mm diameter steel ring was connected to a separate 1500 mm diameter curtain rail by four 600 mm long struts, allowing 360 degree rotation of the cue configuration. Four taut wires fixed across the steel ring were spaced at equidistant points on which objects (cues) were hung. The cues hung approximately 500 mm above the cheeseboard (Figure 2.1).

Figure 2.1. Proximal task setup showing the cue rotation system (Circular curtain rail attached to the ceiling; Steel ring attached by struts; Visual cues).

2.3.1.2. Proximal Cues

A set of five hanging cues were used that varied in size (80 mm – 100 mm wide x 120 mm – 150 mm high), and colour (a red tomato shaped container, a yellow cup with a smiley face, a multicoloured spinning toy, a brown box, and a yellow bath duck) (Figure 2.2). Cues were positioned at 230 mm, 400 mm, 450 mm, 500 mm and 600 mm from the centre of the wire construction (Figure 2.3).

Figure 2.2. Cues used during the proximal task.

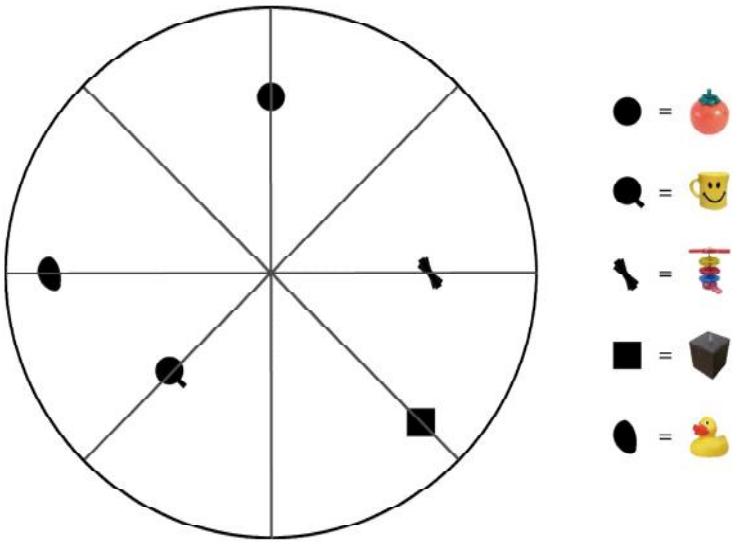

Figure 2.3. Position of cues relative to the centre of the wire construction.

2.3.1.3. Distal Cues

The proximal hanging cues were removed, and the beige curtain was drawn back for this condition. Additional distal cues were provided by a computer and table, holding cage and three-dimensional objects that were fixed to the wall (Figure 2.4).

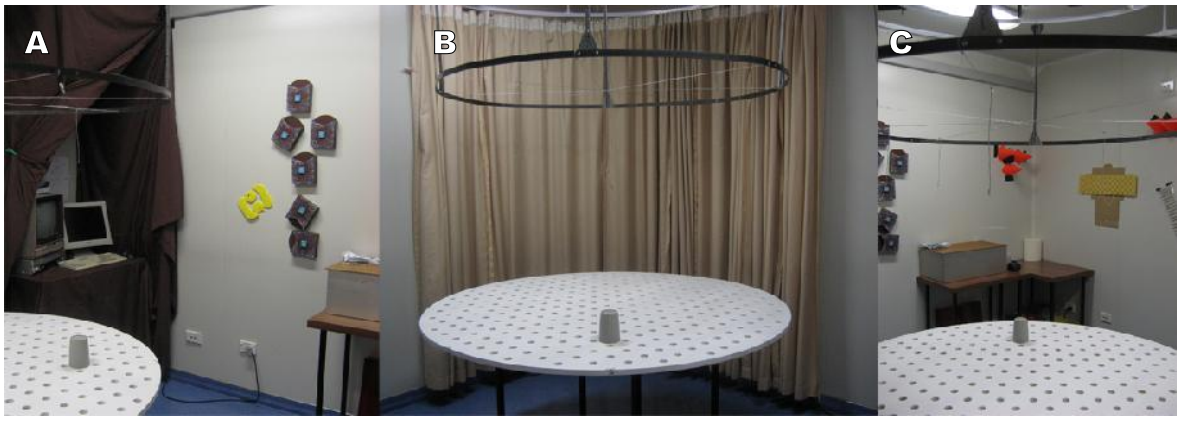

Figure 2.4. Distal task setup showing three views of the visible objects around the testing room. A) The table and computer, B) the curtain C) a selection of 3D objects attached to the wall.

2.3.2. Lighting

The two behavioural tasks were conducted under light conditions. The room was illuminated by overhead fluorescent lights positioned above the centre of the cheeseboard, dimmed to 380 Lux.

2.3.3. Noldus Behaviour Tracking System

A computer located in one corner of the room recorded data acquired during the tasks using Ethovision XT 5.0.212, a video-tracking system (Noldus Information Technology, The Netherlands). To reduce the influence of this noise source, two additional CPUs were run in two of the remaining corners, creating white noise. Data was collected using an infrared video camera fixed to the ceiling, and saved onto standard VHF videotapes to provide backup.

2.4. Behavioural Procedures

For behavioural testing, rats were transported in their cages from their colony room to a holding room adjacent to the experimental room. A single cage of three to four rats was then transferred into the testing room to perform the given task. For any given trial, a rat was gently placed on the table using predetermined start points. Once on the table, the rat was required to search for a food reward. If the food reward was not found within 30 seconds, the rat was guided to it by the experimenter's hand. Once the food reward had been eaten, the rat was removed from the table and placed back into the holding cage.

2.4.1. Pre-surgery Familiarisation

Rats were habituated to search for hidden food rewards (chocolate chips) on the cheeseboard maze. Familiarisation was executed in two phases; 1) group training and 2) individual training (see Table 2.3). The maze was surrounded by a beige curtain to minimise external visual cues.

Table 2.3. Pre-surgery familiarization schedule.

		T_{max}	Release	Beacon	Criterion
Phase 1 Group	Day 1	10 minutes	Centre	No	
	Day 2	5 minutes	Centre	No	
	Day 3	5 minutes	Perimeter	No	
	Day 4	5 minutes	Perimeter	No	
Phase 2 Individual	Day 5	3 minutes	Perimeter	Yes	
	Day 6	3 minutes	Perimeter	Yes	
	Day 7	3 minutes	Perimeter	Yes	90 seconds
Additional	Day 8	unlimited	Perimeter	Yes	90 seconds

Phase one was conducted over four days, with one trial per rat per day. Rats were released in groups of three or four from the centre of the table (day 1 and 2) and then from the perimeter of the table (day 3 and 4). They had to search for hidden food rewards (a few chocolate chips) that were scattered across all of the food wells with no visual cues present. Day 1 was run for 10 minutes and days 2 to 4 were run for 5 minutes.

Phase two was conducted over three days (days 5 to 7) with one trial per rat per day and additional trials for rats that did not locate the food reward within the 3 minute time limit. Each rat was released from one of eight cardinal points (N, E, S, W, NE, NW, SE, or SW) on the perimeter, facing the centre of the table. During each day, rats had a maximum of 3 minutes to locate the food reward (a few chocolate chips) using an adjacent visual beacon (grey beaker, 11 cm high x 7 cm diameter) that was placed on the table behind the food reward, relative to the release point. The location of the food reward and beacon were shifted across days to reduce specific place learning during familiarisation. If by day 7 rats had not located the food reward within a set criterion of 90 seconds, they were run for an eighth day using a trail of chocolate chips that lead from the start point to the beacon to encourage searching. Familiarisation ended once all rats were able to locate the food reward within the 90 second criterion.

2.4.2. Post-surgery Familiarisation

The maze was again surrounded by a beige curtain to minimise external visual cues. Familiarisation was conducted over three days, each with four trials. The maximum time limit was reduced over the three days to optimise performance for behavioural testing (day 1, T_{max} = 120 seconds; day 2, T_{max} = 60 seconds, day 3, T_{max} = 30 seconds).

The rats were required to locate the food reward (three pieces of chocolate in each of the four holes surrounding the beacon) within T_{max} using the grey beacon, as before. Each rat was released from one of eight cardinal points (N, E, S, W, NE, NW, SE, or SW) on the perimeter, facing the centre of the table. The location of the food reward and beacon were shifted across trials to reduce specific place learning during familiarisation. Rats that did not find the food reward were placed next to the beacon for a short period prior to being removed. If at the end of day 3, individual rats had not located the food reward within 30 seconds, at least twice consecutively, additional trials were run until criterion was reached.

2.4.3. Spatial Tasks

Rats were tested in two tasks using either proximal cues or distal cues. These tests included a combination of acquisition and probe days (discussed in subsequent sections) and were designed to measure both egocentric and allocentric navigation strategies, as well as their temporal evolution.

To control for test-order confounds, rats were run in two cohorts (Order 1: Proximal then Distal [PX-DX]; Order 2: Distal then Proximal [DX-PX]). Each cohort was run for two blocks of 28 days, separated by a 28 day rest period.

To control for directional biases, each rat was randomly assigned to either a left or a right food reward location. Irrespective of the specific location of the food reward, the relationship between the food reward (and beacon), the start point and the visual cues remained constant across all acquisition trials. This procedure was designed to allow rats to

learn the relationship between the cues and the food reward as well as the fixed trajectory between the start point and food reward.

2.4.3.1. Proximal Cue Spatial Reference Memory Task

The proximal task involved searching for a hidden food reward (one chocolate chip) on the cheeseboard using a fixed configuration of proximal cues and/or a fixed trajectory (standard configuration: Figure 2.5). Encouragement of the fixed trajectory was established through the placement of a visual beacon behind the food reward and a constant relative start point. The maze was surrounded by a beige curtain to reduce distal cues. In addition, to minimise the use of the ceiling as a cue, each trial was started from a new start point within a day (e.g. N, E, W, S or NE, NW, SW, SE) and the order was changed across days. The release order was pseudorandom and counterbalanced across all days and rats. Irrespective of the start point, the relationship between the start point, food reward and proximal cues remained constant across trials and was achieved by rotating the standard configuration as required. The task was run for 28 days, with four trials per day (total of 112 trials), with a maximum trial duration of 30 seconds. Rats were run in cages of three to four and an inter-trial interval of approximately 5 to 6 minutes. Probe trials were administered during acquisition to assess strategy use via manipulations of the start point and/or cues relative to the food reward location (detailed in Section 2.4.4).

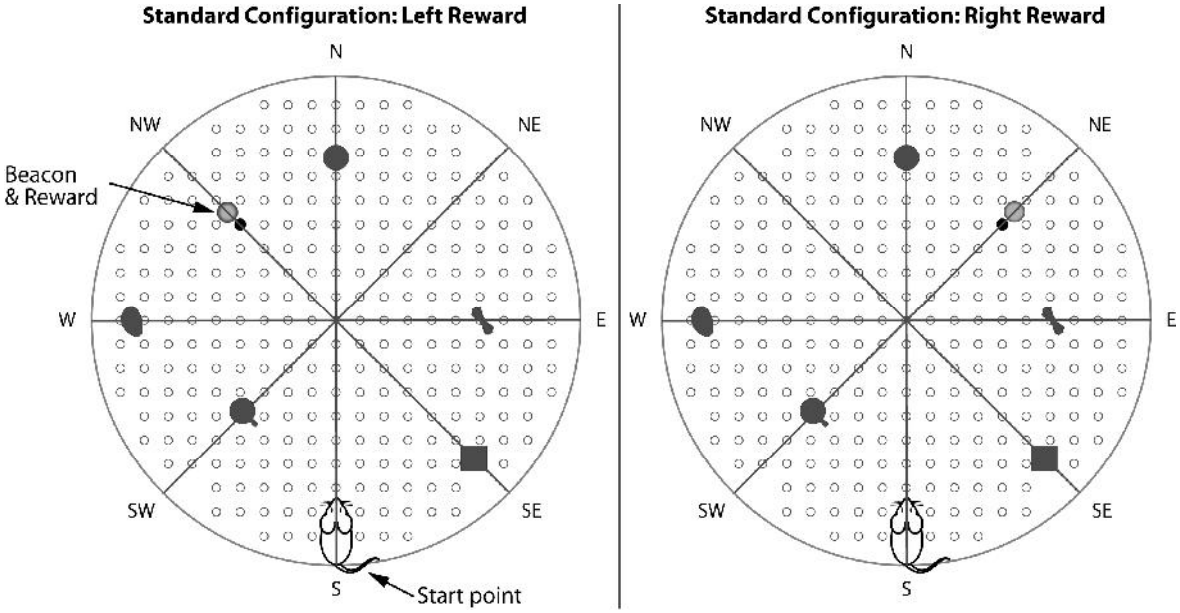

Figure 2.5. Standard configuration. Schematic representation showing the fixed relationship between the five hanging visual cues, beacon/food reward wells (left and right) and start point. The start point could be any of the eight shown, but the cues, beacon and reward stayed constant relative to any start point by rotating the configuration as required. A beige curtain surrounded the cheeseboard to minimise distal cues.

2.4.3.2. Distal Cue Spatial Reference Memory Task

The distal task involved searching for a hidden food reward (one chocolate chip) on the cheeseboard using distal room cues and/or a fixed trajectory. As with the proximal task, use of a fixed trajectory was encouraged by a visual beacon behind the food reward and a constant start position and all other proximal cues were removed. The beige curtain was drawn back and utilised as a static distal cue. Because the distal cues were unable to be rotated, each rat was released from one of eight start points and this remained constant across all acquisition trials. The release points were counterbalanced across all rats. All other procedural details were the same as for the proximal task. For probes, see Section 2.4.4.

2.4.4. Probe Trials: Proximal and Distal Tasks

Three separate probe trials were administered on days 8 – 10, 14 – 16, 20 – 22 and 26 – 28 across the four acquisition weeks (total of 12 probe trials) (see Table 2.4 for details). On

each day, trials 1, 2 and 4 were regular acquisition trials; the third trial involved one of the three probes, as described in Sections 2.4.4.1 to 2.4.4.3.

Table 2.4. Probe testing schedule.

Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1		Acquisition	Acquisition	Acquisition	Acquisition	Acquisition	
2	Acquisition	Acquisition	Probe 1	Probe 2	Probe 3	Acquisition	
3	Acquisition	Acquisition	Probe 1	Probe 2 or 3	Probe 2 or 3	Acquisition	
4	Acquisition	Acquisition	Probe 1	Probe 2 or 3	Probe 2 or 3	Acquisition	
5	Acquisition	Acquisition	Probe 1	Probe 2 or 3	Probe 2 or 3		

The probe trial occurred on the third trial of that day; other trials were regular acquisition trials.

2.4.4.1. Probe 1: General Navigation Probe

To examine the rats' general navigation strategies in the absence of a salient landmark, the beacon was removed from the table leaving the visual cues and start points the same as during acquisition (Figure 2.6). It was necessary to assess behaviour in the absence of the salient landmark as it was an essential manipulation for probes 2 and 3.

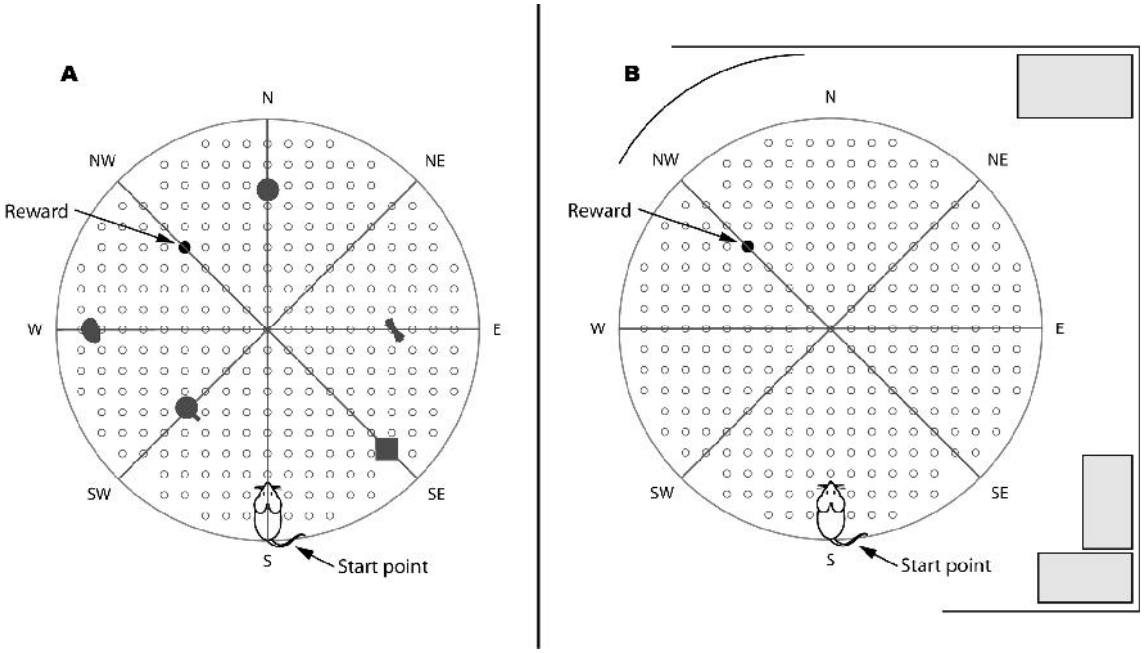

Figure 2.6. Probe 1. A) Proximal setup. B) Distal setup. The beacon was removed from the table, and the start point and visual cues remained standard to assess general navigation in the absence of a salient landmark.

2.4.4.2. Probe 2: Allocentric Probe

The allocentric probe examined whether the rats were able to use the spatial information gained from proximal or distal visual cues in a flexible manner. The beacon was removed from the table and the rat released from a novel start point relative to the standard configuration used during acquisition (Figure 2.7).

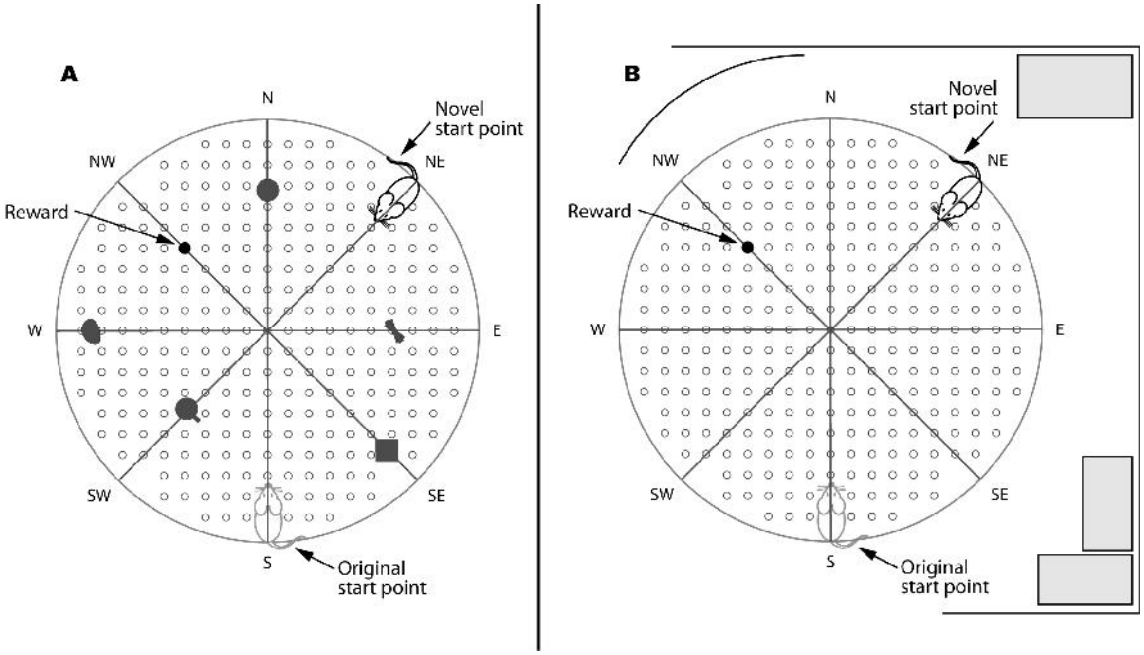

Figure 2.7. Probe 2. A) Proximal setup. B) Distal setup. The beacon was removed from the table and the rat was released from a novel start point to assess the use of allocentric navigation.

2.4.4.3. Probe 3: Egocentric Probe

The egocentric probe examined whether the rats were able to navigate using proprioceptive and vestibular cues when all visual cues were absent. Hence, the beacon and all visual cues were removed but the standard start points were maintained. In the distal task, visual cues were removed by surrounding the maze with the beige curtain (Figure 2.8).

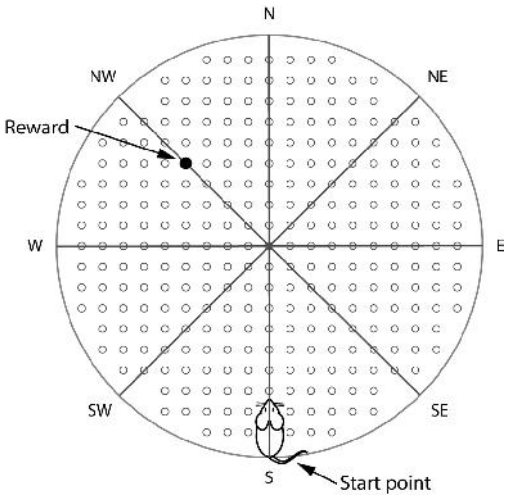

Figure 2.8. Probe 3. The setup for Probe 3 was identical for both proximal and distal tasks. The beacon and all visual cues were removed while the start point remained standard to assess the use of egocentric navigation. In the distal cue task, the local cues were removed by surrounding the maze with the curtain.

2.5. Perfusion

At the completion of behavioural testing the rats were euthanized with sodium pentobarbitone (300 mg/mL) and perfused intracardially (~150 mL saline followed by ~150 mL 4% paraformaldehyde). The brains were post-fixed in 4% paraformaldehyde for a minimum of 7 days, transferred to a long-term sucrose/paraformaldehyde (1% paraformaldehyde in 30% sucrose) solution and stored at 4 degrees Celsius for a minimum of two weeks. Coronal sections (50 μm) through the anterior thalamic nuclei and laterodorsal thalamic nuclei regions were made using a cryostat and stained using cresyl violet (Staining protocol: Appendix I; Table 7.1). Lesion extent was determined by reconstructing the lesion on digitized coronal diagrams (-0.92 mm to -3.60 mm relative to bregma) and lesion size estimated from Paxinos and Watson's rat brain atlas (Paxinos & Watson, 1998) using in-house software. Data for any lesion where the damage sustained did not fall within the specified range (minimum: 35%, maximum: 100%) and/or caused substantial damage to adjacent regions were excluded from subsequent analysis (AT: n=0; LD: n=7). We have previously observed little, if any effects of anterior thalamic lesions when less than 35% in size. Although the intent was to create lesions of 50%, the majority of the laterodorsal thalamic lesions fell between 40% – 50% of the target structure.

3. Results

3.1. Histology

Table 3.1 describes the damage to the anterior thalamic nuclei and to the laterodorsal thalamic nuclei sustained by each rat. The largest and smallest accepted lesions for each lesion group are shown in Figure 3.1 and Figure 3.2.

Table 3.1. Lesion damage analysis for each rat.

AT Lesions

Rat ID	04 D-R	07 H-B	09 J-R	13 O-G	18 D-G	25 M-N	27 P-R	30 F-R	32 J-G	35 L-G	47 Ly-N	56 Te-N	57 Sh-R	58 Ry-R	59 Sl-N	60 Qa-R	62 Ry-B	63 Ui-B
Volume damage (%)																		
AT	**74**	**86**	**80**	**84**	**89**	**97**	**61**	**54**	**96**	**67**	**67**	**56**	**81**	**86**	**64**	**90**	**92**	**54**
LD	6	3	1	0	2	5	1	1	5	0	0	0	0	1	0	1	8	0
LT	6	16	16	9	9	12	21	14	27	9	2	2	4	7	2	8	9	2
MT	1	3	2	2	1	2	1	1	8	2	1	0	1	1	1	1	1	1

LD Lesions

Rat ID	36 A-G	37 B-N*	38 C-R	39 P-B	40 M-G	41 N-B	42 D-B	43 E-B	44 F-G*	45 H-N*	48 D-N*	49 M-B*	50 P-N*	51 I-G	52 O-R	53 O-N	54 N-G*	55 Uc-G
Volume damage (%)																		
AT	1	0	0	0	4	2	3	3	0	0	0	0	0	1	0	0	0	4
LD	**37**	**19**	**76**	**80**	**50**	**40**	**48**	**66**	**23**	**18**	**20**	**2**	**7**	**46**	**48**	**40**	**23**	**47**
LT	0	5	0	0	1	0	1	2	0	1	2	0	0	1	0	0	0	1
MT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Rats excluded from analysis due to damage sustained to the target region being less than 35%; prior experience in our laboratory suggests that small neurotoxic thalamic lesions produce minimal effects.
Abbreviations: AT = anterior thalamic nuclei aggregate comprising the anterodorsal, anteromedial and anteroventral thalamic nuclei; LD = laterodorsal thalamic nuclei aggregate comprising the dorsomedial and ventrolateral nuclei; LT = lateral medial thalamic aggregate comprising the intralaminar nuclei (centrolateral, paracentral and rostral central medial nuclei) and lateral mediodorsal thalamic nuclei (lateral and paralamellar nuclei); MT = posteromedial thalamic nuclei aggregate comprising the central and medial mediodorsal nuclei and the intermediodorsal nucleus.

3.1.1. Lesion Evaluation

Only data for the rats that sustained damage of 35% or more to the target region were analysed. The following description provides the mean damage sustained and the range across rats, shown in brackets. The AT lesion group sustained damage to 76.52% (53.77% - 96.54%) of the target AT region, with only 1% (0% - 7.57%) damage to the LD region. The LD lesion group sustained damage to 52.51% (37.43% - 79.66%) of the target LD region, with only 1.76% (0% - 4.10%) damage to the AT region. There were no differences in lesion size between the PX-DX and DX-PX task orders for the AT lesion group (Order, $F_{(1,25)} = 2.08$, NS) or the LD lesion group (Order, $F_{(1,25)} = 0.03$, NS).

In the AT lesion group, damage sustained to adjacent regions include the stria terminalis 40.66% (0.26% - 77.01%), central medial thalamic nucleus (rostral) 10.86% (0.04% - 32.87%), interanteromedial thalamic nucleus 42.50% (14.90% - 70.97%), mediodorsal thalamic nucleus 14.84% (0% - 26.71%), mediolaterodorsal thalamic nucleus 21.37% (0.09% - 45.25%), paracentral thalamic nucleus 18.5% (7.48% - 35.65%), reticular thalamic nucleus 10.15% (5.42% - 17.77%), submedius thalamic nucleus 9.37% (0% - 79.75%), ventral anterior thalamic nucleus 26.34% (2.60% - 57.70%) and the ventromedial thalamic nucleus 5.90% (0.14% - 35.29%).

In the LD lesion group, damage sustained to adjacent regions include the angular thalamic nucleus 40.16% (0% - 57.60%), laterorostral thalamic nucleus 4.21% (0% - 16.77%), mediorostral thalamic nucleus 7.81% (0% - 29.02%), posterior thalamic nucleus 53.99% (0.20% - 100%), ventral anterior thalamic nucleus 4.70% (0% - 25.50%), ventrolateral thalamic nucleus 10.42% (0.26% - 30.52%), ventral lateral geniculate nucleus 1.80% (0% - 11.28%) and the ventral posteromedial thalamic nucleus 1.60% (0% - 13.29%).

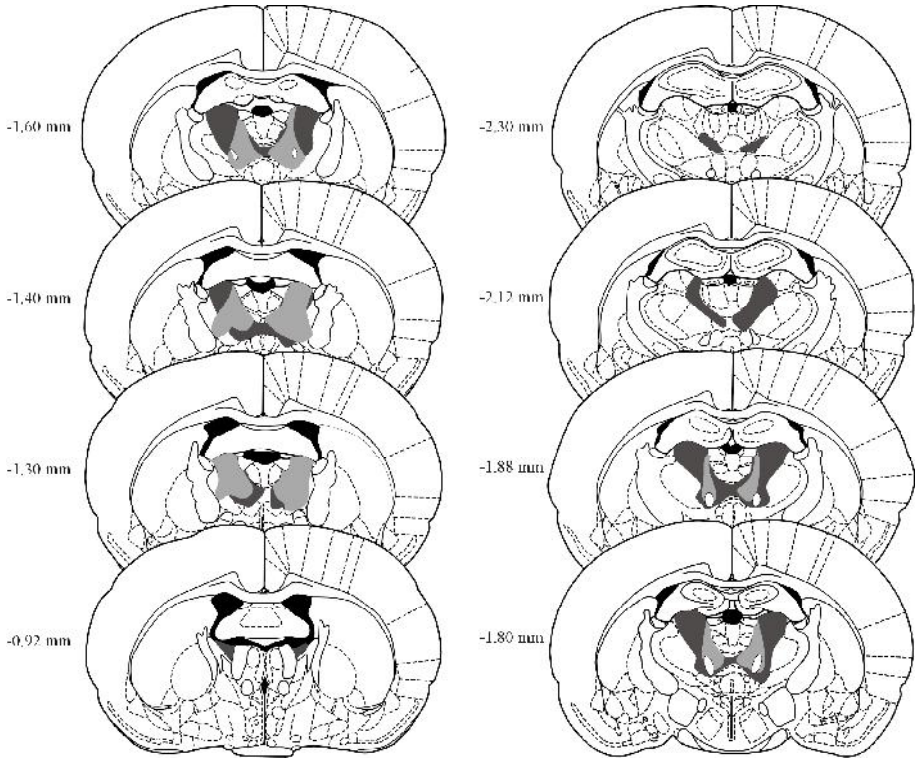

Figure 3.1. Schematic coronal sections through the anterior thalamic region (-0.92 mm to -2.30 mm relative to bregma) superimposed with maximum (dark grey) and minimum (light grey) lesion sizes. Schematics are adapted from Paxinos and Watson, (1998).

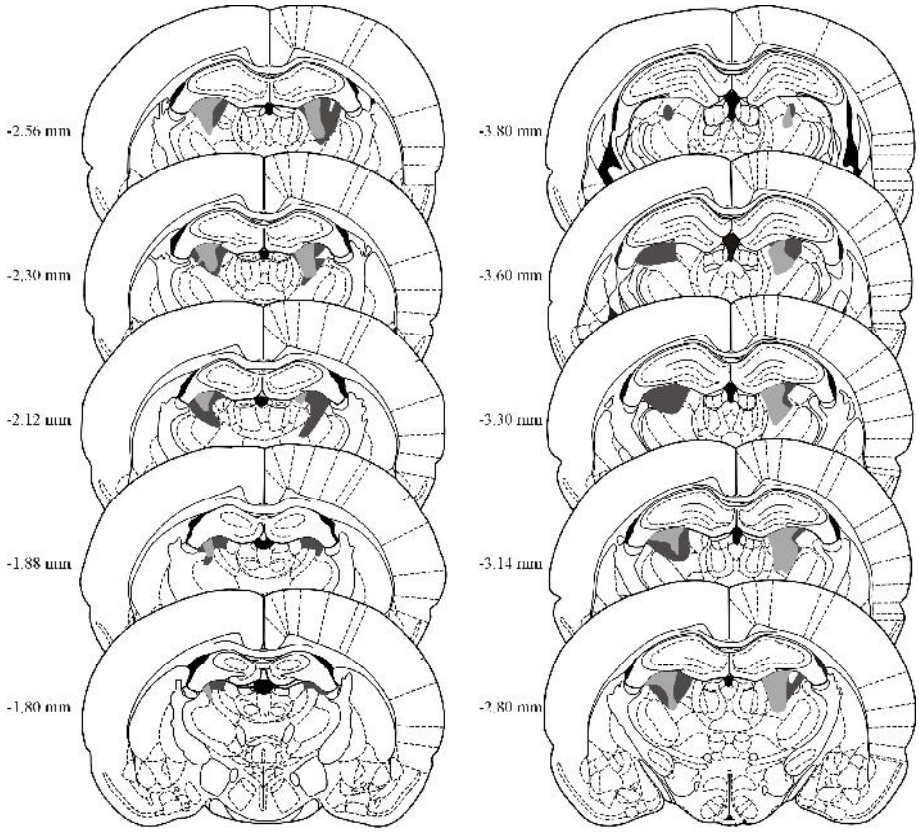

Figure 3.2. Schematic coronal sections through the laterodorsal thalamic region (-1.80 mm to -3.80 mm relative to bregma) superimposed with maximum (dark grey) and minimum (light grey) lesion sizes. Schematics are adapted from Paxinos and Watson, (1998).

3.2. Behavioural Testing

Navigation accuracy was measured using the deviation from a direct path to the food reward (deviation scores) and time taken to locate the reward location (latency).

The deviation scoring system, adapted from Whishaw, Hines & Wallace, (2001), is shown in Figure 3.3. Each rat received a single deviation score for each trial, which was based on the number of times each zone was entered. For example, if a rat took a direct route to the food reward, it received a score of zero. If it crossed into zone '1' twice and zone '3' twice, the total deviation score would be eight (1x2 + 3x2).

Figure 3.3A shows the 'standard' scoring system used for all acquisition trials and all probes. A 'modified' scoring system was created for probe 2 only (Figure 3.3B and C) in which additional zones were created enabling both allocentric and egocentric deviation scores to be calculated through data transposition. The shaded areas were scored as follows: dark grey = '0', mid grey = '1' and light grey = '3'.

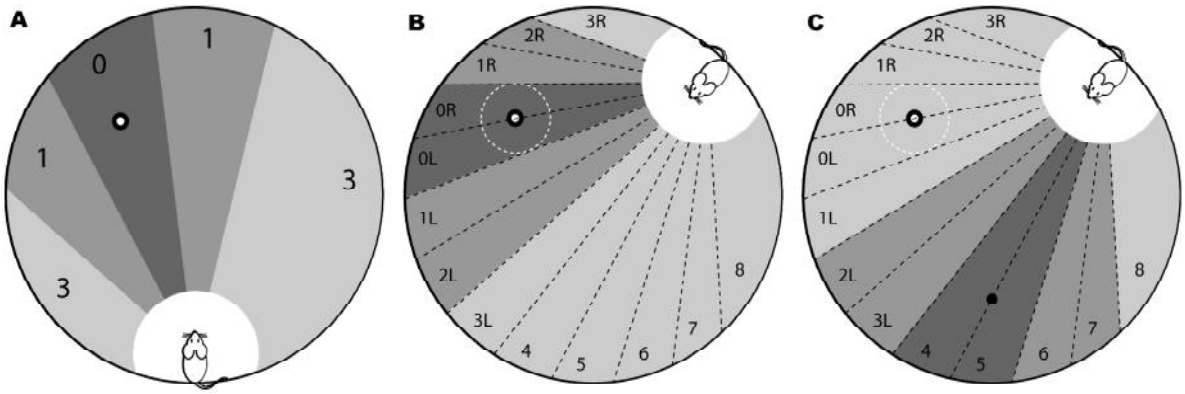

Figure 3.3. A) Standard deviation scoring system for navigation accuracy to the food reward (indicated by the white circle). B/C) Modified scoring system used in probe 2 only to enable deviation scores to be calculated through data transposition for both allocentric (B) and egocentric (C) strategies from a single probe trial. The expected food reward location based on egocentric strategies is indicated by the black dot. White dotted circles represent the place location criterion for probe trials. A rat that entered the zone indicated by '0' received a score of zero representing accurate heading direction with little or no deviation (dark grey). If it crossed into zones '1' (mid grey) or '3' (light grey) it received that score each time it crossed into that zone. The scores were then totaled for a single deviation score for each trial.

Deviation scores assessed in probe trials are only a measure of the heading direction (discussed in Section 3.3), not specifically whether the rat located the food reward (place

learning). Instead, this was measured by latency, using a lenient place location criterion compared to acquisition (see Figure 3.3B/C).

3.2.1. Corrections for Missing Data

Due to occasional procedural errors when recording run paths in Ethovision, a number of trials were not recorded. To analyse the full data set in Statistica, missing data from acquisition trials were estimated for each rat using the average of a single session for that rat (Missing trials: N=32; 0.004% of acquisition data). Missing data from acquisition trials that occurred on probe days were analysed for each rat using the average of a given trial (e.g. trial 1) across weeks for that rat (Missing trials: N=38; 0.010% of probe acquisition data). Missing data from probe trials were calculated using a multiple regression of the adjacent two weeks (Missing trials: N=29; 0.018% of probe data). For example, if datum for a single rat was missing in WK2, then the data of all rats in WK1 and WK3 were used to predict the score for WK2 given the rats own performance on WK1 and WK3 (Statistica).

3.2.2. Comparison of Sham Lesions

3.2.2.1. Proximal Task

A 2 x 2 x 15 repeated measures analysis of variance (ANOVA) was conducted to determine whether differences in deviation scores across acquisition days were present between the two sham lesion groups (sAT and sLD) and the task order (PX-DX and DX-PX). No main effects or interactions were found between type of sham Lesion or Order ($F < 1.0$), therefore the data from the sham groups were pooled and analysed as a single sham group.

3.2.2.2. Distal Task

A 2 x 2 x 15 repeated measures analysis of variance (ANOVA) was conducted to determine whether differences in deviation scores across acquisition days were present

between the two sham lesion groups (sAT and sLD) and the task order (PX-DX and DX-PX). No main effects or interactions were found between type of sham Lesion or Order ($F < 1.0$), therefore the data from the sham groups were pooled and analysed as a single sham group.

3.2.3. Spatial Reference Memory Task: Acquisition

3.2.3.1. Proximal Task

Rats were required to learn to find a food reward that was located in front of a grey beacon by using the available proximal visual cues and/or a fixed trajectory. Irrespective of the strategy used by a rat to find the food reward during acquisition, it was expected that the deviation scores would reduce across days as the animals learnt the task. To reduce the effect of task novelty, data from day 1 have been excluded. Acquisition refers to the five blocks of three days that did not include probe trials (days 2 – 4, 5 – 7, 11 – 13, 17 – 19 and 23 – 25). No lesion effects were expected across the acquisition trials, as work by Save and Poucet (2000) showed that rats with either hippocampal or parietal cortex lesions (presumed to be comparable to AT and LD lesions, respectively) could learn to navigate accurately toward a salient beacon.

Figure 3.4 shows an overall decrease in deviation scores in the proximal task across acquisition (3-day blocks) for all three lesions groups (AT, LD and sham) (Block effect, $F_{(4, 164)} = 80.05$, $p < 0.001$). This indicates that the rats learnt to accurately navigate towards the location of the food reward when all visual cues were available. There was also a Lesion effect ($F_{(2,41)} = 5.46$, $p < 0.001$) with poorer performance in the AT group. However, performance was affected by the order in which the task was undertaken. Rats that performed the proximal task first had higher deviation scores (poorer performance) than rats that had had prior training in the distal task (Order effect, $F_{(1,41)} = 17.10$, $p < 0.001$; Block x Order interaction ($F_{(4,164)} = 4.16$, $p < 0.001$). Relative to the sham and LD lesion groups, the AT lesion group demonstrated a more pronounced order effect with

higher deviation scores, although the Lesion x Order interaction just failed to reach significance ($F_{(2,41)} = 2.84$, $p = 0.07$; Lesion x Block x Order, $F < 1.0$).

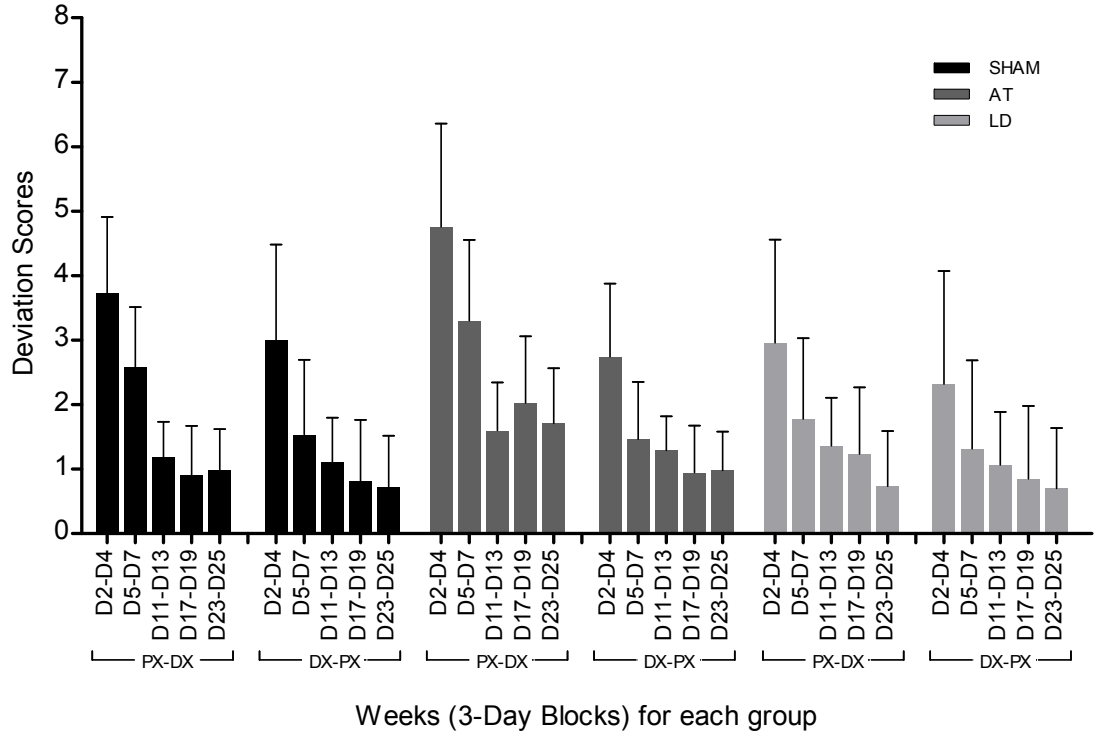

Figure 3.4. Proximal task, Acquisition. Mean change in deviation scores (±SEM) over acquisition in the proximal task across groups. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

Figure 3.5 shows that the performance of the AT lesion group was worse in the PX-DX condition than in the DX-PX condition. They were also worse than the sham and LD lesion groups in the proximal task when tested in the PX-DX order. A *post-hoc Bonferroni* test confirmed that the AT lesion group had better performance in the proximal task after completing the distal task first than did the AT lesion group that started in the proximal task first (adjusted $p < 0.001$). As discussed in Section 3.1.1, this effect was not due to any differences in lesion size between the task orders.

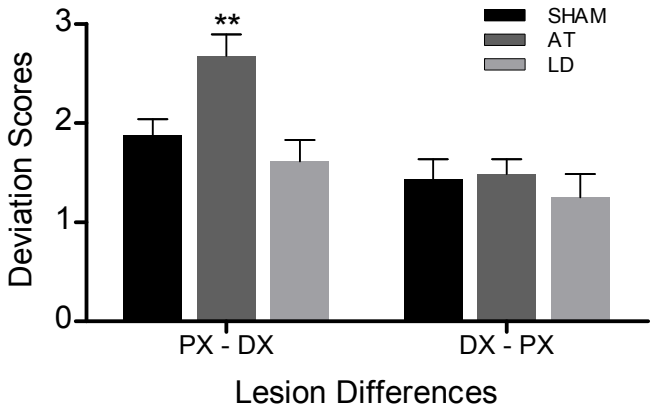

Figure 3.5. Proximal task, Acquisition. Mean deviation score (± SEM) for each lesion group across acquisition for the two task orders. The AT lesion group in the PX-DX condition showed significantly higher deviation scores compared to the sham and LD lesion group, but this was not present in the DX-PX condition. ** Indicates statistical significance of $p < 0.001$. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

In the proximal task, the latency score measured how long it took rats to locate the food reward when the beacon and proximal visual cues were available to guide navigation during acquisition. Figure 3.6 shows a decrease in latency across acquisition for all three lesion groups (Block effect, $F_{(4,164)} = 31.15$, $p < 0.001$), which is consistent with deviation score data. There was no Lesion effect ($F < 1.0$) and despite the LD lesion group taking longer to find the food reward when no other training had occurred, neither the Order effect ($F_{(1,41)} = 3.46$, $p = 0.07$), nor the Lesion x Order interaction ($F_{(2,41)} = 2.61$, $p = 0.09$) reached significance. A *post-hoc Bonferroni* test confirmed that the LD lesion group did not differ significantly from the either the sham or AT lesion group in the proximal task when tested in the PX-DX order. There was no Block x Lesion or Block x Order interaction (Fs < 1.3).

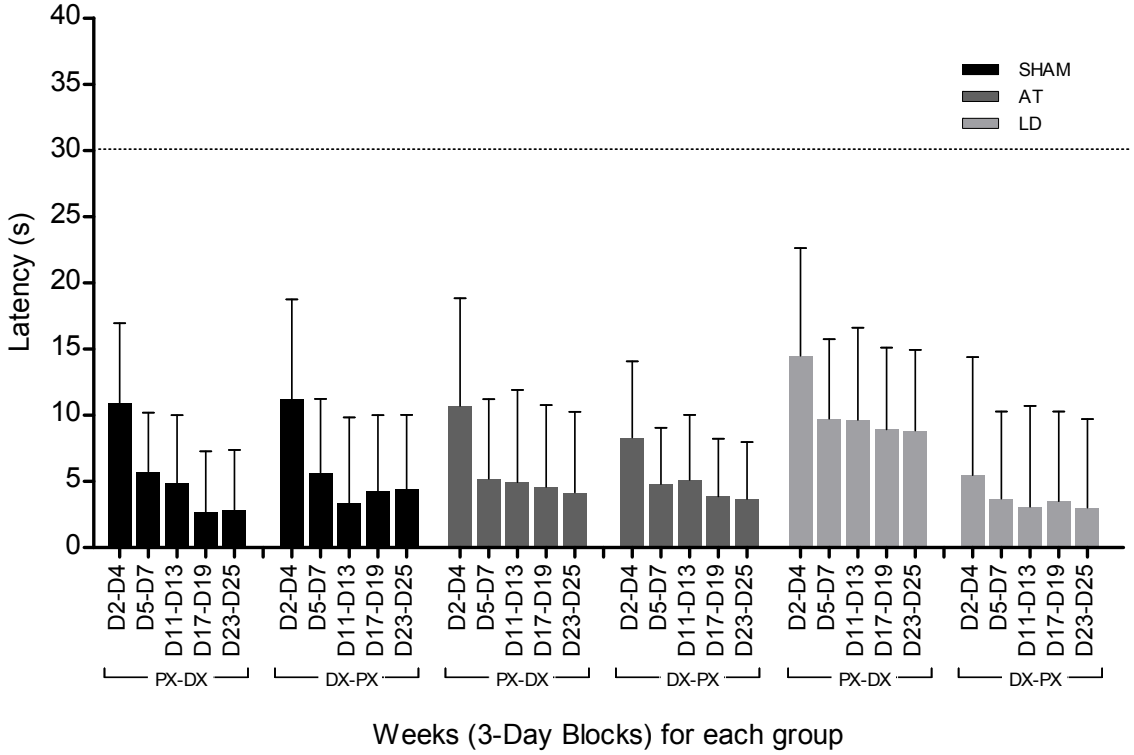

Figure 3.6. Proximal task, Acquisition. Mean change in latency (±SEM) to locate the food reward across acquisition in the proximal task across groups. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.2.3.2. Distal Task

Rats were required to learn to find a food reward that was located in front of a grey beacon by using the available distal visual cues and/or a fixed trajectory. All other procedural details were the same as for the proximal task.

Figure 3.7 shows an overall decrease in deviation scores in the distal task across acquisition (five blocks of three days: 2 – 4, 5 – 7, 11 – 13, 17 – 19 and 23 – 25) for all three lesion groups (Block effect, $F_{(4,164)} = 20.08$, $p < 0.001$). This is consistent with the proximal task, in which rats learnt to navigate accurately when all visual cues were available. While performance in the proximal task was affected by the task order, with better performance when prior testing had occurred, no differences were seen in the distal task (Order effect, $F < 1.0$). However, a Block x Order interaction was significant ($F_{(4,164)} = 3.11$, $p < 0.05$), but this was in large part influenced by the high deviation scores of the AT lesion group in the first three days of training (Lesion x Block x Order, $F < 1.5$; post-

hoc Bonferroni test, adjusted $p < 0.05$). Compared to the sham and LD lesion groups, performance across both order conditions was consistently poorer in the AT lesion group (Lesion effect, $F_{(2,41)} = 4.56$, $p < 0.05$; Lesion x Order interaction, $F < 1.0$) (Figure 3.8).

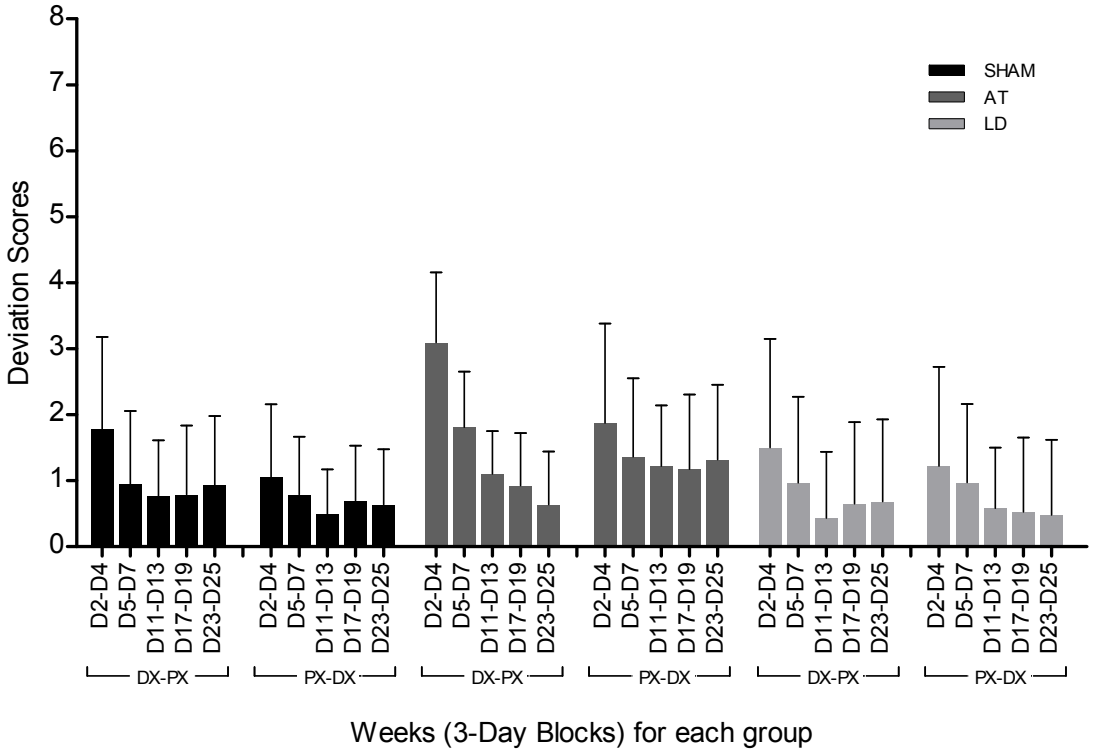

Figure 3.7. Distal task, Acquisition. Mean change in acquisition deviation scores (±SEM) over acquisition in the distal task across groups. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

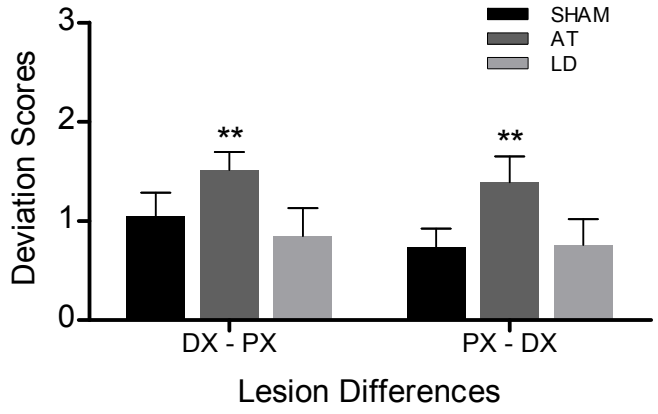

Figure 3.8. Distal task, Acquisition. Mean deviation score (± SEM) for each lesion group across acquisition tasks for the two task orders. The AT lesion group showed significantly higher deviation scores compared to the sham and LD lesion groups in both task orders. ** Indicates statistical significance of $p < 0.001$. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

In the distal task, the latency score measured how long it took rats to locate the food reward when the beacon and distal visual cues were available to guide navigation during acquisition. Figure 3.9 shows an decrease in latency across acquisition (Block effect, $F_{(4,164)} = 7.38$, $p < 0.001$) which is consistent with deviation score data. Performance across acquisition was poorer when prior training had occurred (Block x Order interaction, $F_{(1,41)} = 6.03$, $p < 0.001$; Order effect, $F_{(1,41)} = 2.51$, $p = 0.12$). The sham and LD lesion groups, but not the AT lesion group, took longer to find the food reward when prior training had occurred in the proximal task, but this did not reach statistical significance (Lesion x Order interaction, $F_{(1,41)} = 2.54$, $p = 0.09$; Lesion x Block x Order, $F < 1.0$). A *post-hoc Bonferroni* test showed that there were no significant differences between the three lesion groups in the distal task when tested in the PX-DX order. There was no Lesion effect or Block x Lesion interaction (Fs < 1.0).

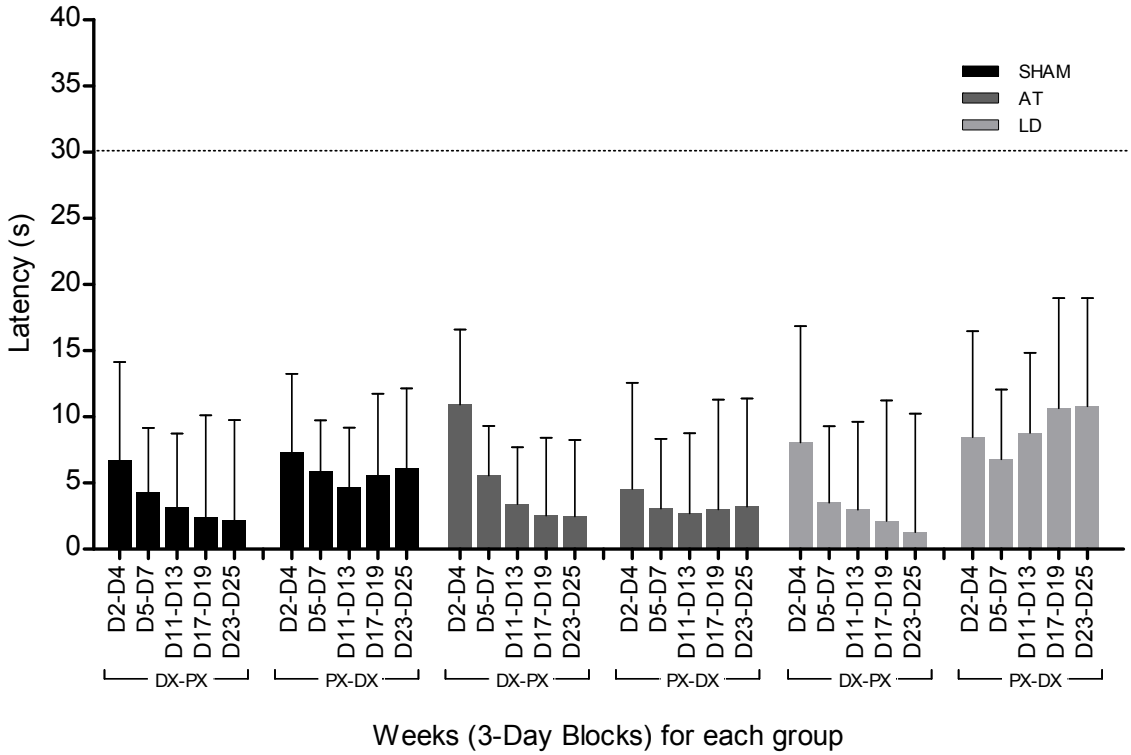

Figure 3.9. Distal task, Acquisition. Mean change in latency (±SEM) to locate the food reward over acquisition in the distal task across groups. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.2.4. Comparison of Acquisition: Proximal versus Distal Cues to Guide Navigation

To assess how the proximity of visual cues affected navigation with the beacon and visual cues intact, data have been collapsed across order and lesions. As seen in Figure 3.10, both deviation scores (especially) and latency are higher in the proximal conditions compared to distal conditions. Accuracy was disrupted substantially across acquisition when proximal cues guided navigation (Task effect, $F_{(1,44)} = 24.70$, $p < 0.001$; Block effect, $F_{(4,176)} = 80.90$, $p < 0.001$; Task x Block interaction, $F_{(4,176)} = 12.45$, $p < 0.001$). However, latency to locate the food reward was not disrupted when proximal cues guided navigation (Task effect, $F_{(1,44)} = 1.98$, NS; Task x Block interaction, $F_{(4,176)} = 1.39$, NS). A planned comparison between the proximal and distal task conditions in the first week of training (D2-D4) just failed to reach significance ($F_{(1,44)} = 3.39$, $p = 0.07$). This suggests that initially, rats spent longer searching for the food reward when proximal cues guided navigation, but this effect was ameliorated with prolonged training. As rats learnt the task, latency decreased across acquisition for both task conditions resulting in a significant Block effect ($F_{(4,176)} = 42.08$, $p < 0.001$). These results indicate that the proximity of the visual cues had an effect on navigation accuracy, and had little influence on latency, when all other variables were held constant.

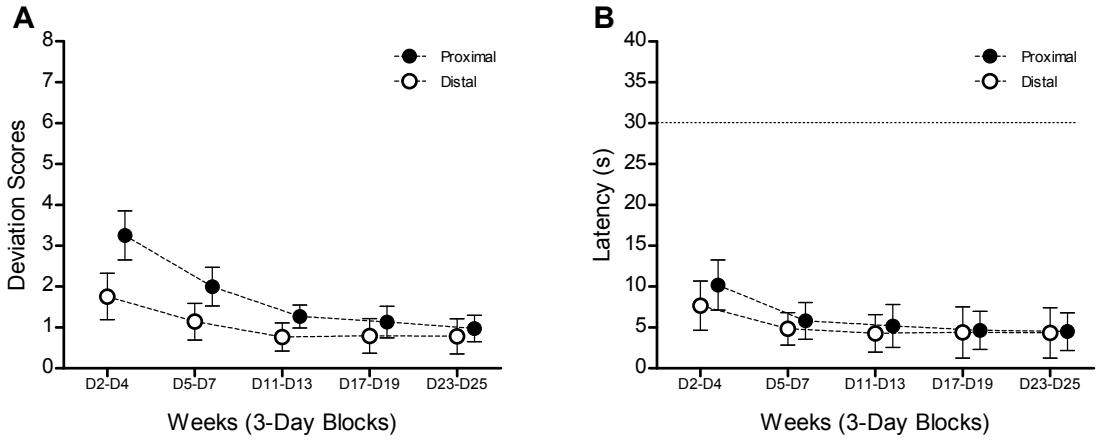

Figure 3.10. Proximal versus Distal task, Acquisition. A) Comparison of mean change in deviation scores (±SEM) over acquisition. B) Comparison of mean change in latency (±SEM) to locate the food reward over acquisition. Navigation accuracy was consistently poorer across acquisition in the proximal task condition. Latency was initially poorer in the proximal task condition, but was similar to the distal task condition at asymptote.

3.3. Probe Trials

Three separate probe trials were administered on each of the four probe weeks to examine egocentric and allocentric navigation strategies over time. This was achieved in the first probe by removing the beacon, while leaving the visual cues and start points in the standard setup. In the second probe, the beacon was removed and the start point was novel relative to the standard configuration. In the third probe all visual cues were removed but the standard start points were maintained. Rats were given 30 seconds to search for the food reward in each probe trial; however, the initial navigation decision was the important feature. To measure this initial navigation, the distance run was averaged across all non-probe acquisition trials that occurred on probe days and two standard deviations were added to this distance to determine cut-offs (proximal cut-off: 158 cm; distal cut-off: 140 cm). The deviation scores were then calculated based on these maximum distance values.

3.3.1. Acquisition Trials versus Probe-Acquisition Trials

A number of systematic analyses were performed to determine which "acquisition" trial or trials were the most appropriate to compare against the corresponding probe trials.

3.3.1.1. Proximal Task: Week 1 – 4; ACQ T1, T2, T4 versus pACQ T1, T2, T4

To check that there were no significant differences between standard acquisition trials (ACQ) and acquisition trials that occurred on probe days (pACQ) in the proximal task, the first (T1), second (T2) and fourth (T4) trials were analysed across weeks. The third trial (T3) was omitted as it was always a probe trial on probe days.

Figure 3.11 shows that the deviation scores for all trials in the first week prior to the introduction of probe trials across both ACQ and pACQ were higher than the successive three weeks. This indicates that learning was still taking place during week 1 and performance had not reached asymptote. This was supported by a significant Week effect ($F_{(3,123)} = 25.78$, $p<0.001$) and a *post-hoc Bonferroni* test confirmed that week 1 was significantly different from weeks 2, 3 and 4 (adjusted $p < 0.05$). Variability can also be

seen between T1, T2 and T4 with higher deviation scores observed in T4 compared to T1 and T2. This was supported by a Trial effect ($F_{(2,82)} = 14.82$, $p < 0.001$) and a *post-hoc* *Bonferroni* test confirmed that T4 was significantly different from T1 and T2 (adjusted, $p < 0.05$). Overall, there were no significant differences between ACQ and pACQ (Task effect ($F < 1.30$), but there was a Task x Week interaction ($F_{(3,123)} = 3.51$, $p < 0.05$). The first week and T4 were therefore excluded in the following analyses.

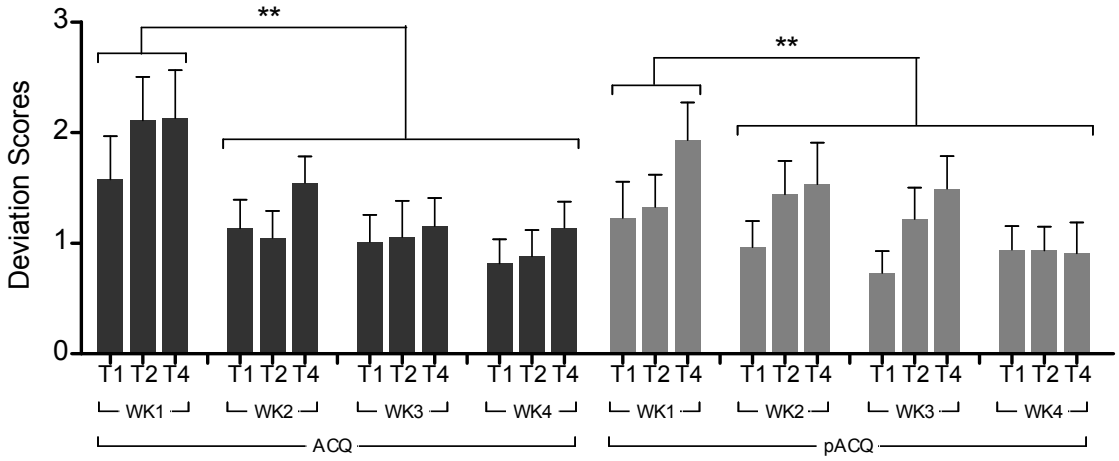

Figure 3.11. Proximal task, Acquisition. Acquisition trials (ACQ) versus acquisition trials that occurred on probe days (pACQ). Overall, no significant differences were observed between ACQ and pACQ. However, week 1 differed significantly from subsequent weeks and T4 differed significantly from T1 and T2. ** Indicates statistical significance of $p < 0.001$.

3.3.1.2. Proximal Task: Week 2 – 4; ACQ T1, T2 versus pACQ T1, T2

Because the deviation scores were higher and more variable in Week 1 and T4 in the previous analysis, only T1 and T2 for weeks 2 to 4 were compared.

Most evident in Figure 3.12 is the variability in deviation scores between T1 and T2 in the pACQ trials, compared to greater stability between T1 and T2 in ACQ. This was supported by a significant Task x Trial interaction ($F_{(1,41)} = 5.69$, $p < 0.05$). The Trial effect just failed to reach significance ($F_{(1,41)} = 3.66$, $p = 0.06$). There was no Task effect ($F < 1.0$) or Task x Week interaction ($F < 1.0$), but there was an effect of Week ($F_{(2,82)} = 4.10$, $p < 0.05$) which suggests that performance continued to improve across training.

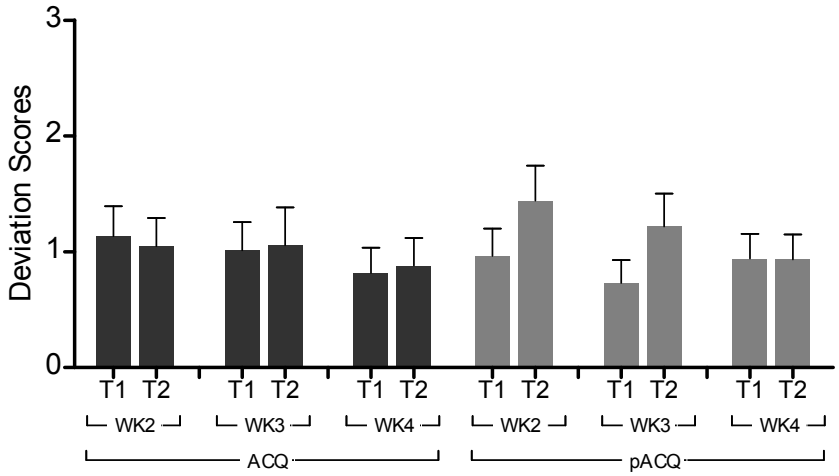

Figure 3.12. Proximal task, Acquisition. Comparison of T1 and T2 across weeks. Acquisition trials (ACQ) versus acquisition trials that occurred on probe days (pACQ). There was more variability between T1 and T2 in pACQ compared to ACQ.

3.3.1.3. Proximal Task: Week 2 – 4; ACQ T2 versus T3

Recall that T3 was omitted from analysis in Section 3.3.1.1 as it was always a probe trial on probe days. However, because of the greater variability in deviation scores on T2 in pACQ, not seen in ACQ trials or pACQ T1, the following analysis compared trials T2 and T3 within ACQ only. Thus, the following analysis was to make sure there were no systematic differences between the two trials due to trial order. The absence of systematic differences would provide evidence that differences between T3 ACQ and the probes were genuine. There were no significant differences in deviation scores across Weeks ($F < 1.2$) or Trials ($F_{(1,41)} = 3.55$, $p = 0.07$) for T2 and T3 within ACQ only (Figure 3.13). Therefore, T3 data from ACQ were pooled (ACQ.T3) and provided a reasonable estimate to compare against all probe trials (which were always the third trial on probe days).

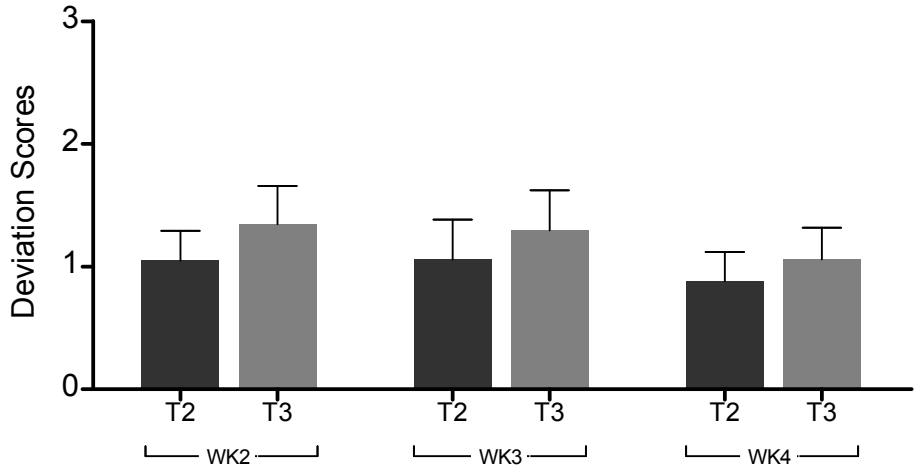

Figure 3.13. Proximal task, Acquisition. Comparison of ACQ T2 and T3 across weeks. There were no significant differences between T2 and T3.

3.3.1.4. Distal Task: Week 1 – 4; ACQ T1, T2, T4 versus pACQ T1, T2, T4

The same analytic sequence as the proximal task was used to establish the most appropriate ACQ trials to compare with the probe trials in the distal task, starting with T1, T2 and T4 across ACQ and pACQ.

Figure 3.14 shows that the performance was slightly poorer in the first week, with higher deviation scores than the subsequent three weeks. This indicates that learning was still taking place during week 1 and performance had not reached asymptote. This was supported by a significant Week effect ($F_{(3,123)} = 6.30$, $p < 0.001$) and a *post-hoc Bonferroni* test confirmed that week 1 was significantly different from weeks 2, 3 and 4 (adjusted $p < 0.001$) in the ACQ condition. Week 1 was only significantly different from week 4 in the pACQ condition ($p < 0.05$). There was no effect of Task ($F < 1.2$) or Trial ($F < 1.0$).

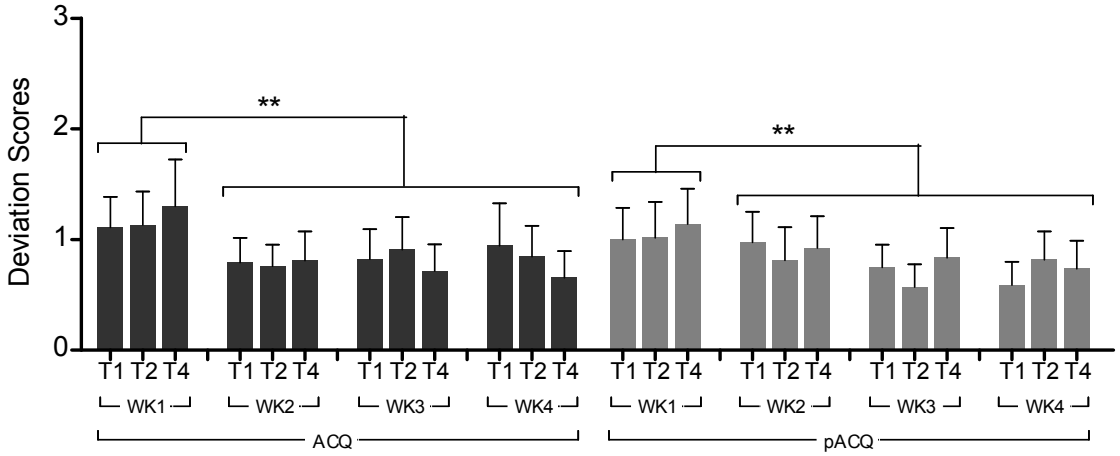

Figure 3.14. Distal task, Acquisition. Acquisition trials (ACQ) versus acquisition trials that occurred on probe days (pACQ). Overall, no significant differences were observed between ACQ and pACQ trials. However, week 1 differed significantly from subsequent weeks. ** Indicates statistical significance of $p < 0.001$.

3.3.1.5. Distal Task: Week 2 – 4; ACQ T1, T2 versus pACQ T1, T2

While no Trial effects were present in the prior analysis, the first week and T4 were excluded from the subsequent analysis to ensure consistency across proximal and distal tasks. There were no discernible differences between ACQ and pACQ across weeks or trials (Figure 3.15). The Task effect just failed to reach significance ($F_{(1,41)} = 3.57$, $p = 0.07$), and there was no effect of Week or Trial and no Week x Trial interaction (Fs < 1.0).

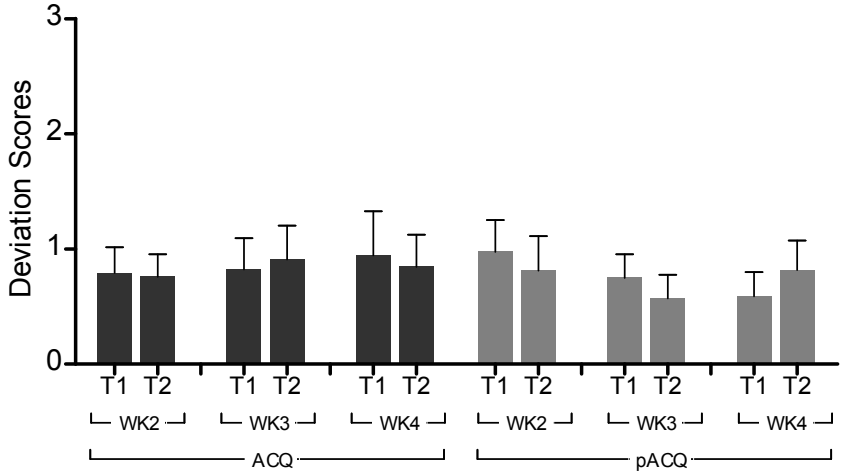

Figure 3.15. Distal task, Acquisition. Comparison of T1 and T2 across weeks. Acquisition trials (ACQ) versus acquisition trials that occurred on probe days (pACQ).

3.3.1.6. Distal Task: Week 2 – 4; ACQ T2 versus T3

This analysis was conducted to ensure consistency between proximal and distal tasks. T3 was omitted from analysis in Section 3.3.1.4 as it was always a probe trial on probe days. Hence, T2 and T3 were compared to make sure there were no systematic differences between these two ACQ trials due to trial order. The absence of systematic differences would provide evidence that differences between ACQ and probes were genuine. There were no significant differences in deviation scores across Weeks ($F < 1.0$) or Trials F < 2.5) (Figure 3.16). Therefore, T3 data from ACQ were pooled (ACQ.T3) and provided a reasonable estimate to compare against all probe trials (which were always the third trial on probe days).

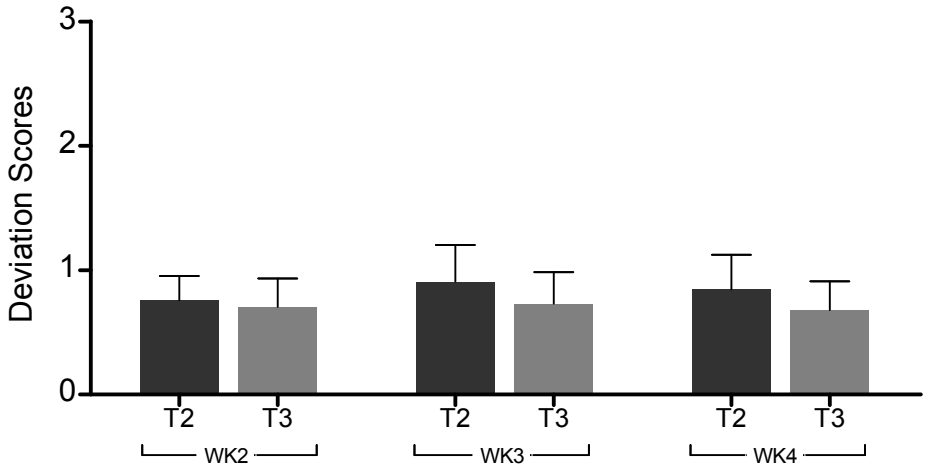

Figure 3.16. Distal task, Acquisition. Comparison of ACQ T2 and T3 across weeks. There were no significant differences between T2 and T3.

3.3.2. Probe 1: General Navigation Probe

Table 3.2. Probe 1 Parameters.

Beacon	Start Point	Visual Cues
Removed	*Standard (no change)*	*Standard (no change)*

3.3.2.1. Proximal Task (Probe 1)

Probe 1 was always run on the first of the three probe days across each of the four probe weeks. General navigation strategies were assessed by removing the beacon, and leaving the visual cues and start points in the standard setup (Table 3.2 and Figure 3.17). This was expected to produce an increase in deviation scores across all lesion groups as the beacon was part of the cue-matrix and provided spatial information, but because the remaining visual cues were still available, the disruption, at least in sham lesioned rats, was not expected to be large.

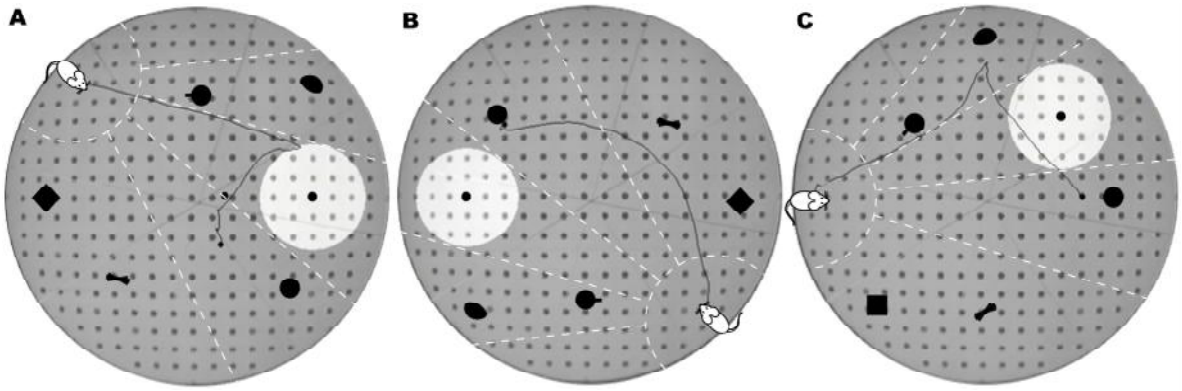

Figure 3.17. Proximal task, Probe 1: General navigation. Examples of run paths during probe trials. A) Sham (score = 2); B) AT (score = 4); C) LD (score = 2).

Figure 3.18A shows the performance of rats in the proximal task across four weeks of probe trials (P1 WK1 – P1 WK4), in which only the beacon was removed, compared to acquisition (ACQ.T3). A marked increase in deviation scores was observed across the four probe trials compared to ACQ.T3 (Task effect, $F_{(4,164)} = 7.21$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 69.62$, $p < 0.001$), but not from each other. Thus, rats were more dependent on the beacon than the spatial configuration of proximal cues to

navigate towards and locate the food reward. Furthermore, Figure 3.17 shows that when the salient beacon was removed, instead of using the proximal cue configuration to build a spatial representation to locate the food reward, rats tended to use one or more remaining cues to guide navigation. Figure 3.18B shows the probe trial data from Figure 3.18A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.0$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

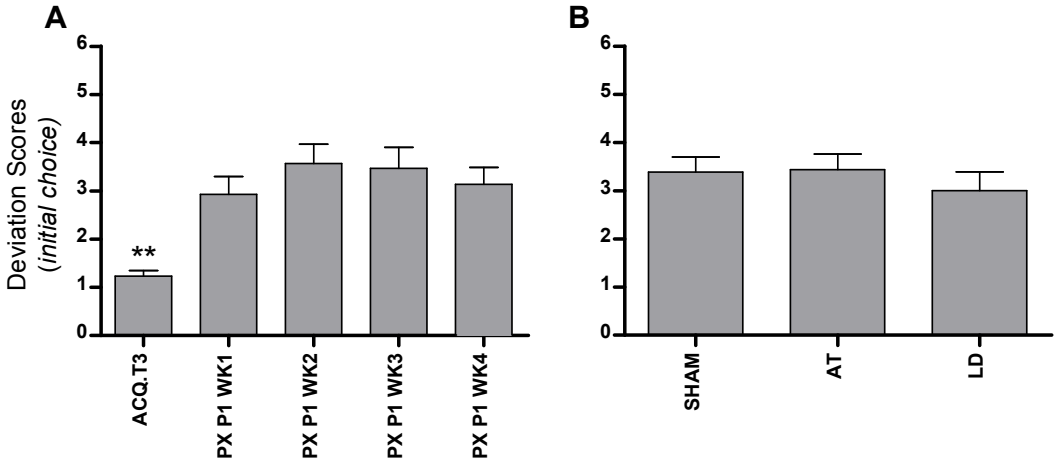

Figure 3.18. Proximal task, Probe 1: General navigation. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed with standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of $p < 0.001$.

Figure 3.19 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$) and across Weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 2.60).

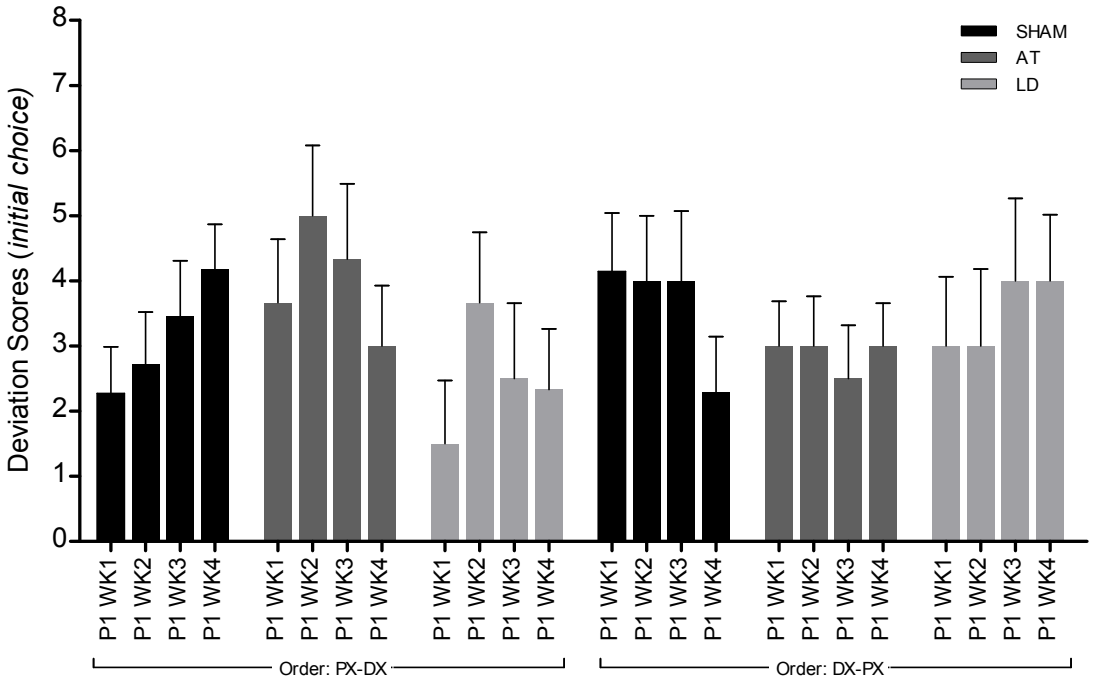

Figure 3.19. Proximal task, Probe 1: General navigation. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

Figure 3.20 shows the latency for each lesion group to locate the food reward in probe trials compared to acquisition (ACQ.T3) across task order conditions and weeks. There was a marked increase in latency in the probe trials compared to acquisition trials (ACQ.T3) (Task effect, $F_{(4,164)} = 8.26$, $p < 0.001$). A planned comparison confirmed that all probe trials differed significantly from acquisition ($F_{(1,41)} = 80.42$, $p < 0.001$), but not from each other. This indicates that rats failed to use the proximal cue configuration to locate the food reward when the beacon was removed from the cue matrix. Comparing only the probe trials, there were no effects of Lesion or Order (Fs < 1.1), nor any Lesion x Order, Week x Lesion or Week x Order interactions (Fs < 1.5).

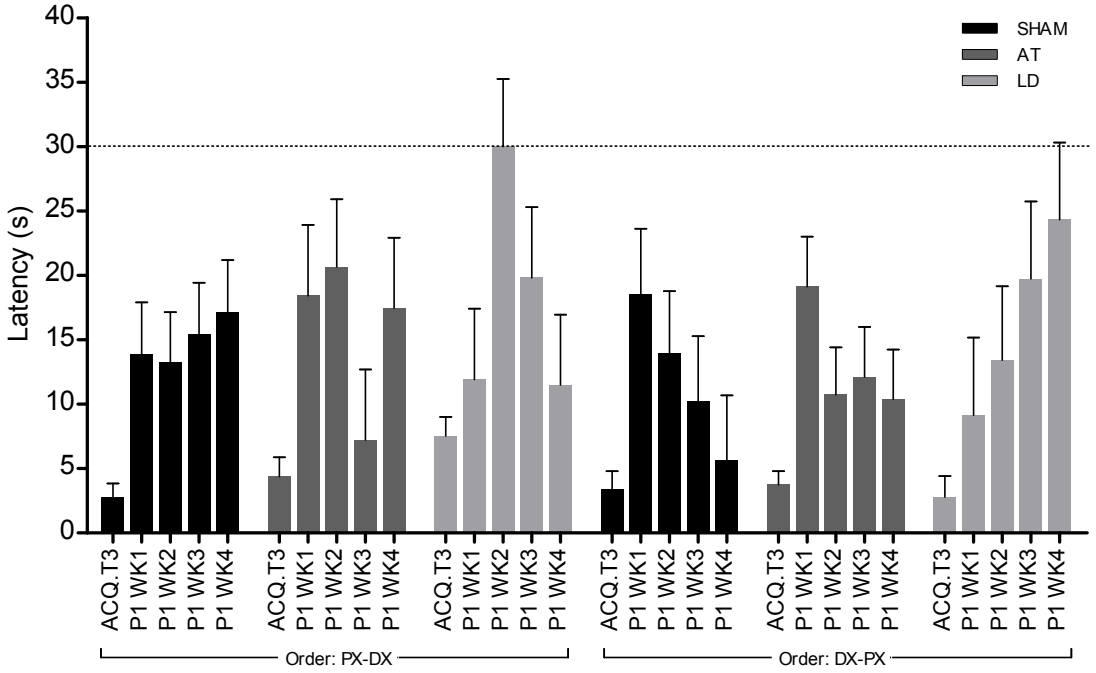

Figure 3.20. Proximal task, Probe 1: General navigation. Comparison of probe trials (WK1 – WK4; beacon removed with standard configuration intact) versus ACQ.T3 (beacon and standard configuration intact). Mean latency (±SEM) to locate the food reward across task order and weeks for each lesion group. Compared to acquisition, latency increased for all three lesion groups. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.2.2. Distal Task (Probe 1)

As per the proximal task, general navigation strategies were assessed by removing the beacon and leaving the visual cues and start points in the standard setup (Table 3.2 and Figure 3.21). This was expected to produce a small increase in deviation scores across all lesion groups as the beacon provided spatial information, but because the remaining visual cues were still available, the disruption, at least in sham lesioned rats, was not expected to be large.

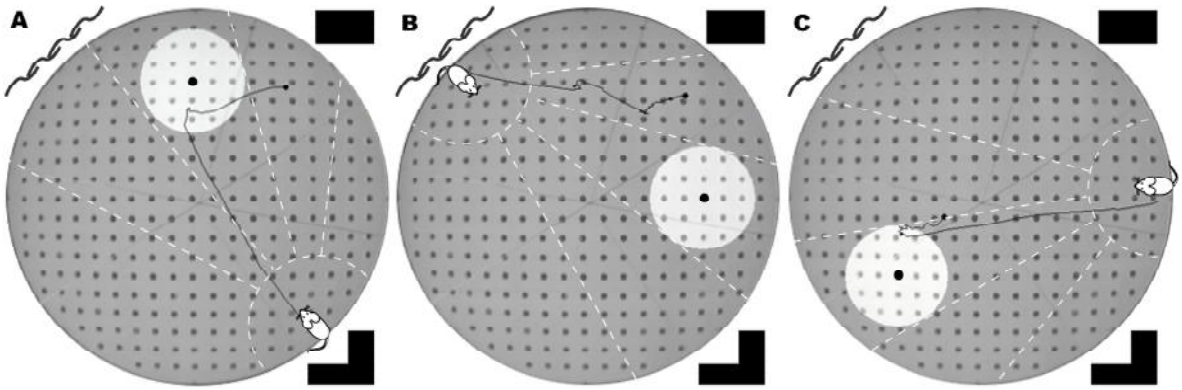

Figure 3.21. Distal task, Probe 1: General navigation. Examples of run paths during probe trials. A) Sham (score = 2); B) AT (score = 1); C) LD (score = 1). The distal cues represent the curtain and salient room cues, but are not drawn to scale.

Figure 3.22A shows the performance of rats in the distal task across four weeks of probe trials (P1 WK1 – P1 WK4), in which only the beacon was removed compared to acquisition (ACQ.T3). A small increase in deviation scores was observed across the four probe trials compared to ACQ.T3. Although the overall Task effect was not significant ($F_{(4,164)} = 1.73$, p = 0.15), the planned comparison revealed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 24.39$, $p < 0.001$), but not from each other. An increase in deviation scores across the probe trials relative to acquisition indicates that the beacon helped guide navigation, but disruption to the heading direction when removed was not as marked as it had been in the proximal task. This was supported by the running paths in Figure 3.21 which shows the rats were able to use the distal cue configuration to facilitate a more accurate heading direction. Figure 3.22B shows the probe trial data from Figure 3.22A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, when performance of the sham group was not severely disrupted, a lesion effect was evident, with the AT lesion group performing worse than the LD and sham lesion groups (Lesion effect, $F_{(1,41)} = 4.58$, $p < 0.05$). A planned comparison confirmed that the AT lesion group differed from the sham lesion group ($t = 2.64$, $p < 0.05$) and the LD lesion group ($t = 2.53$, $p < 0.05$) and that there was no difference between the LD and sham lesion groups.

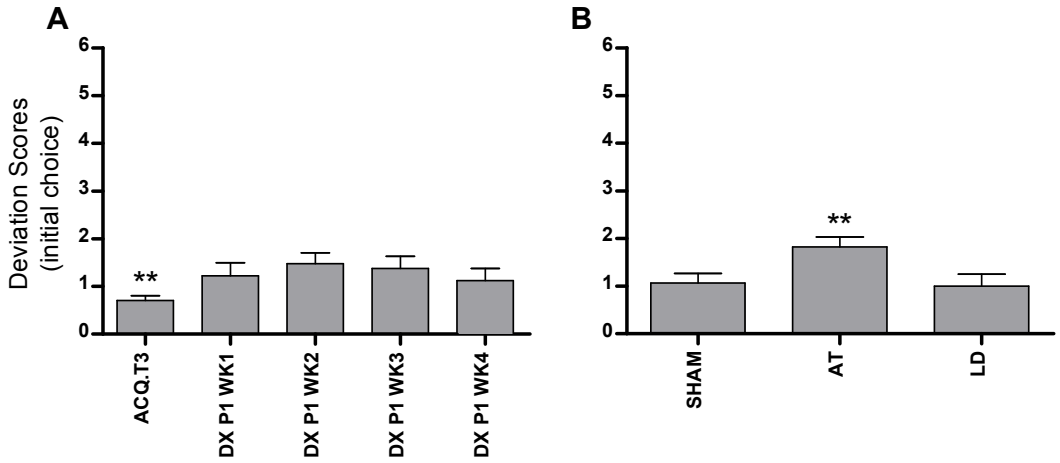

Figure 3.22. Distal task, Probe 1: General navigation. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed with standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) Significant differences were seen between the lesion groups across the four probe trials (WK1 – WK4), with the AT lesion group showing the poorest performance compared to the sham and LD lesion groups. There were no differences between the sham and LD lesion groups. ** Indicates statistical significance of $p < 0.001$.

Figure 3.23 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$) and across Weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 1.0).

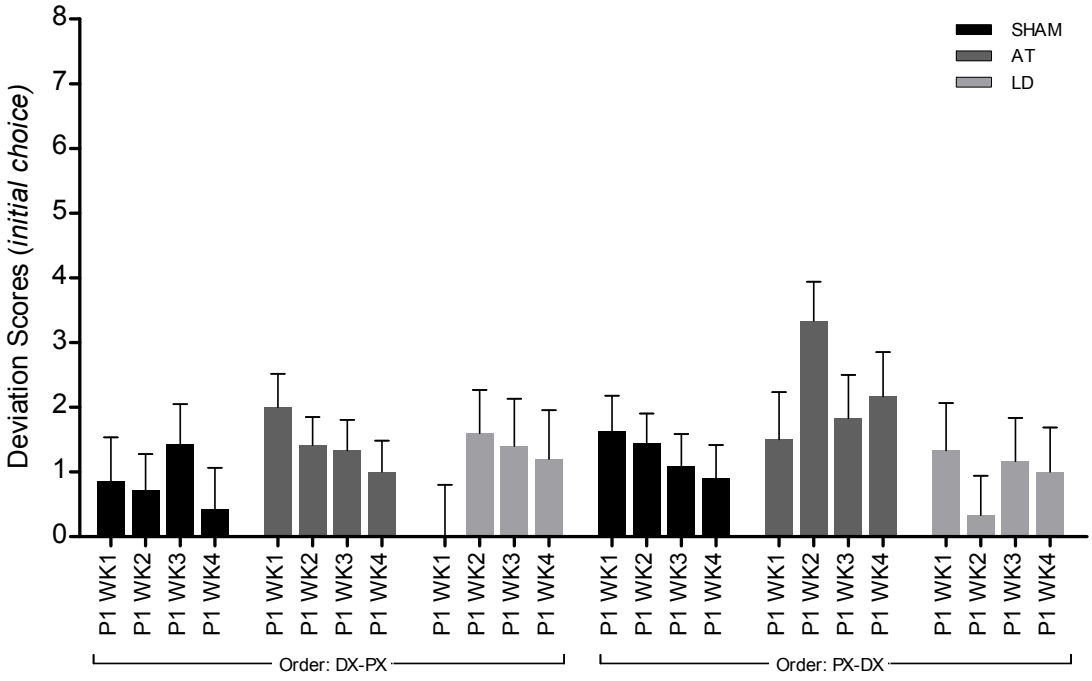

Figure 3.23. Distal task, Probe 1: General navigation. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

Figure 3.24 shows the latency for each lesion group to locate the food reward in probe trials compared to acquisition (ACQ.T3) across task order conditions and weeks. There was no significant overall Task effect ($F_{(4,164)} = 1.84$, $p = 0.12$), but a planned comparison confirmed that all probe trials differed significantly from acquisition ($F_{(1,41)} = 21.26$, $p < 0.001$), but not from each other. This indicates that rats were disrupted, but still able to use the distal cues to locate the food reward when the beacon was removed from the cue matrix. Comparing only the probe trials, there was no effect of Lesion ($F < 1.0$). Clear differences can be seen between the task order conditions in LD group, but a planned comparison just failed to reach significance ($t = 1.85$, $p = 0.07$). Latency appeared longer when the rats had had prior training, but the Order effect just failed to reach statistical significance ($F_{(1,41)} = 3.22$, $p = 0.08$). There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 1.6).

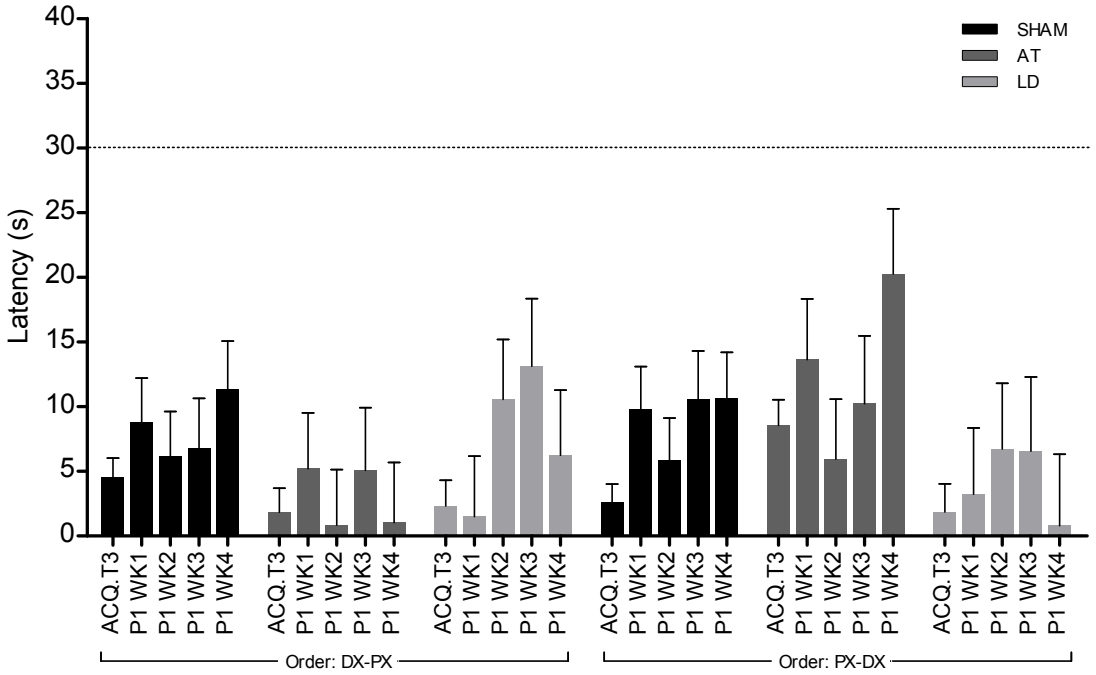

Figure 3.24. Distal task, Probe 1: General navigation. Comparison of probe trials (WK1 – WK4; beacon removed with standard configuration intact) versus ACQ.T3 (beacon and standard configuration intact). Mean latency (±SEM) to locate the food reward across task order and weeks for each lesion group. Compared to acquisition, latency increased for all three lesion groups. No effects of week or lesion were seen and the order effect just failed to reach significance. The difference in performance between task orders in the LD group just failed to reach significance. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.3. Probe 2: Allocentric Probe

Table 3.3. Probe 2 Parameters (Allocentric).

Beacon	Start Point	Visual Cues
Removed	*Novel*	*Standard (no change)*

3.3.3.1. Proximal Task (Probe 2 – Allocentric measure)

Two sets of analyses will be discussed in this section. The first follows the same 'standard' scoring system used for acquisition, probe 1 and probe 3 (Figure 3.25A). The second is a 'modified' scoring system which emphasises the accuracy of navigation from a novel start point and is derived from further divisions of each scoring zone (Figure 3.25B/C).

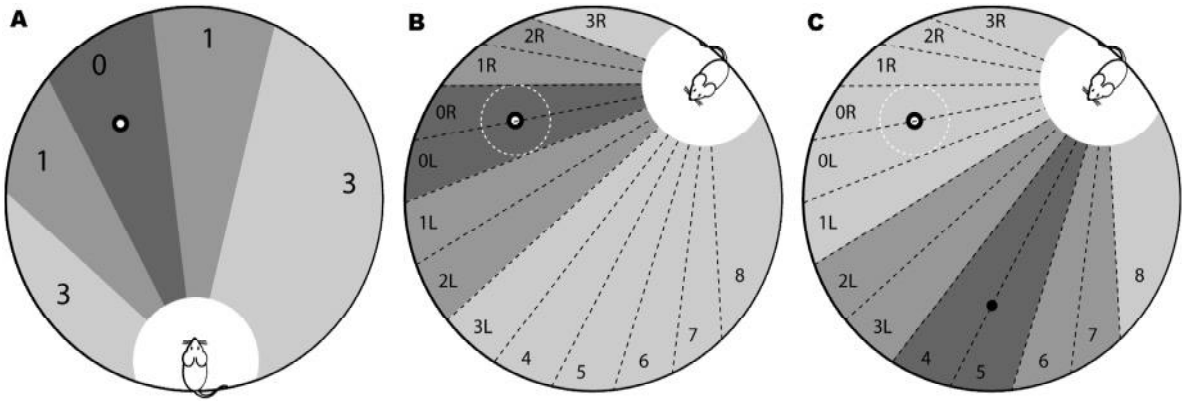

Figure 3.25. Illustration of the standard and modified scoring systems utilized for the analysis of probe 2. A) Each zone was scored as stated. To emphasize navigation accuracy, zones were divided for an allocentric measure (B) and an egocentric measure (C). Each zone shaded in dark grey was scored as '0', each zone shaded in mid grey was scored as '1' and each zone shaded in light grey was scored as '3'.

To assess whether rats were able to use spatial information in a flexible manner (allocentric navigation strategy), the standard configuration was maintained but the rat was released from a novel start point and the beacon was removed (Table 3.3 and Figure 3.26). Based on current literature, it was expected that AT lesioned rats would show the greatest impairment because allocentric strategies needed to be employed, whereas LD lesioned rats would show an intermediate impairment compared to sham rats. It was expected that sham lesioned rats would exhibit slightly poorer performance compared to acquisition, as the task was considered more difficult.

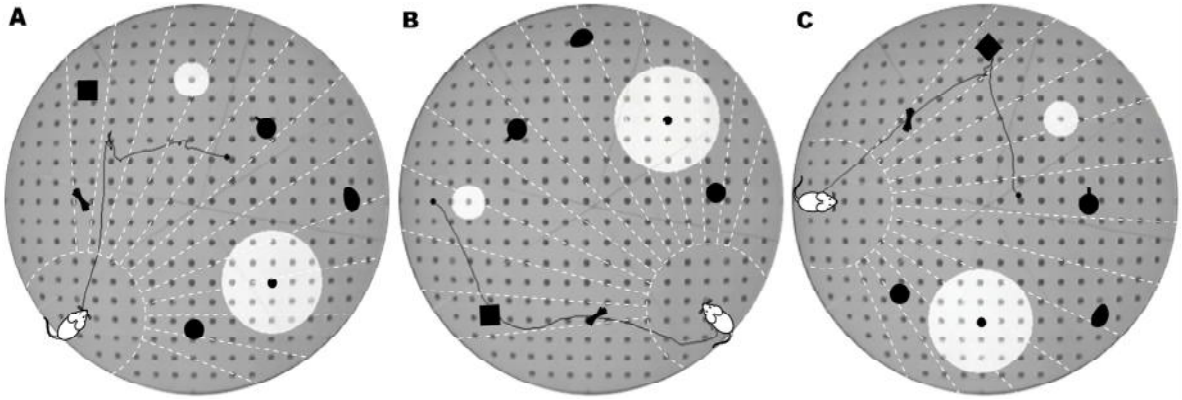

Figure 3.26. Proximal task, Probe 2: Allocentric measure. Examples of run paths during probe trials. A) Sham (standard score = 3; modified score = 12); B) AT (standard score = 3; modified score = 12); C) LD (standard score = 3; modified score = 14).

Figure 3.27A shows the performance of rats in the proximal task across four weeks of probe trials (P2a WK1 – P2a WK4) when released from a novel start point compared to acquisition (ACQ.T3). A marked increase in deviation scores was observed across the four probe trials compared to ACQ.T3 (Task effect, $F_{(4,164)} = 29.49$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 148.49$, $p < 0.001$), but did not differ from each other. This suggests that when released from a novel start point, rats failed to use the spatial relationship of the proximal cues to guide navigation. This is supported by the running paths shown in Figure 3.26. The paths clearly show the initial heading trajectory is based on the learned trajectory (toward the small circle), suggesting the use of egocentric strategies. In addition to using an egocentric heading direction, rats tended to use one or more remaining cues to guide navigation, instead of using the overall spatial configuration gained from the proximal cue matrix to locate the food reward. Figure 3.27B shows the probe trial data from Figure 3.27A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.0$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

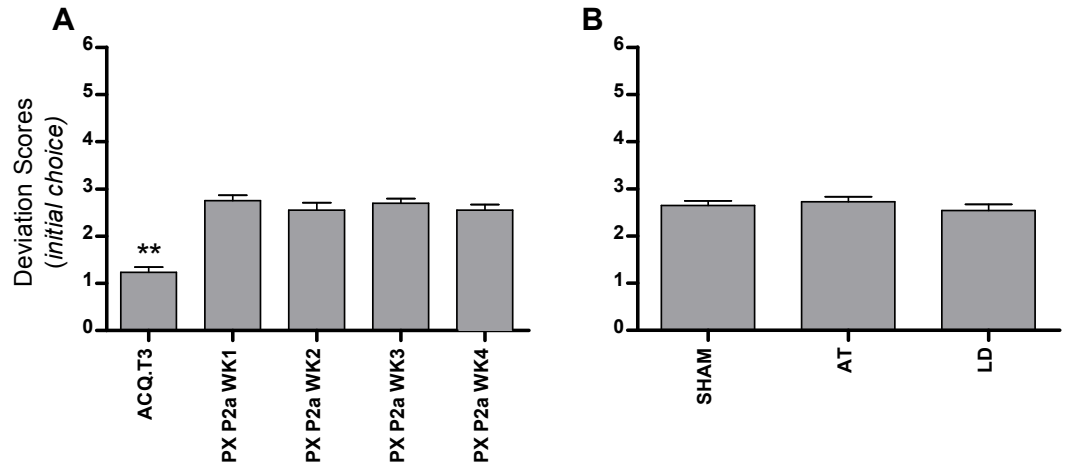

Figure 3.27. Proximal task, Probe 2: Allocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of $p < 0.001$.

Figure 3.28 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) and for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$) and Weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction ($F < 1.0$).

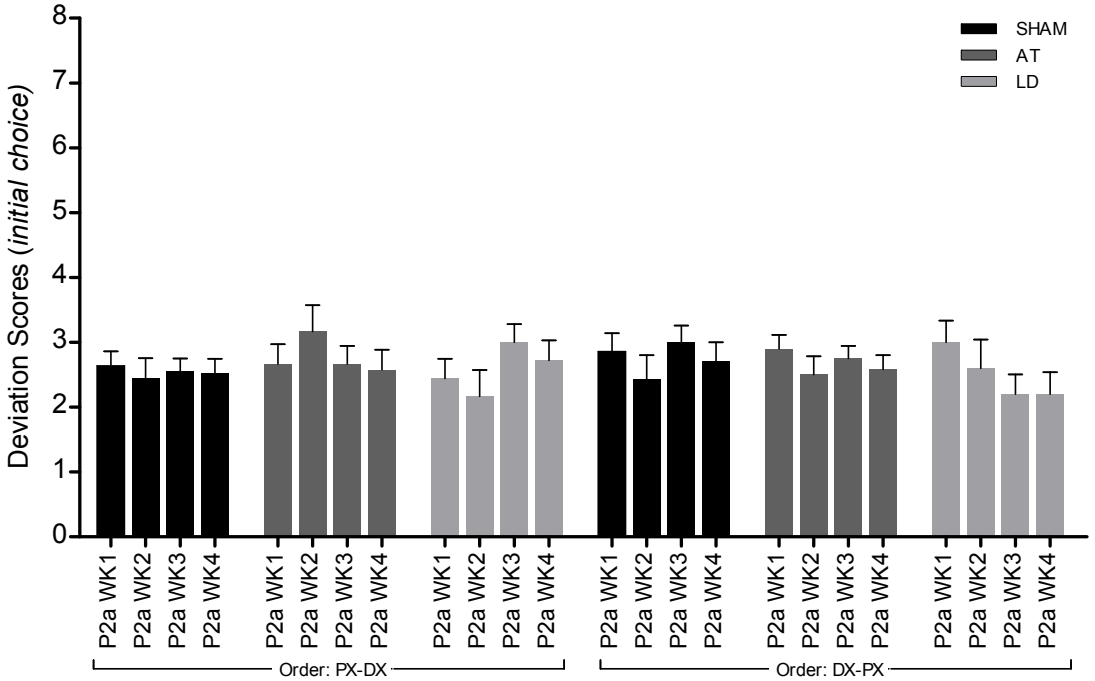

Figure 3.28. Proximal task, Probe 2: Allocentric measure. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

Figure 3.29 shows the latency for each lesion group to locate the food reward in probe trials compared to acquisition (ACQ.T3) across order conditions and weeks. There is a substantial increase in latency in the probe trials compared to acquisition trials (ACQ.T3) (Task effect, $F_{(4,164)} = 112.05$, $p < 0.001$), with mean latencies often close to the maximum trial duration (30 seconds). A planned comparison confirmed that all probe trials differed significantly from acquisition ($F_{(1,41)} = 826.57$, $p < 0.001$), but not from each other. The high latency scores reflect that the rats often did not locate the food reward area when released from a novel start. Comparing only probe trials, there was a difference between the lesion groups (Lesion effect, $F_{(2,41)} = 3.05$, $p = 0.06$). A planned comparison showed that the sham group performed worse than the LD lesion group ($t = 2.32$, $p < 0.05$), and the AT lesion group did not differ from either the sham or LD lesion groups. There was no effect of Order ($F < 1.0$), nor was there a Lesion x Order or Week x Lesion interaction (Fs < 1.0). There was, however, a Week x Order interaction ($F_{(3,123)} = 2.59$, $p = 0.06$).

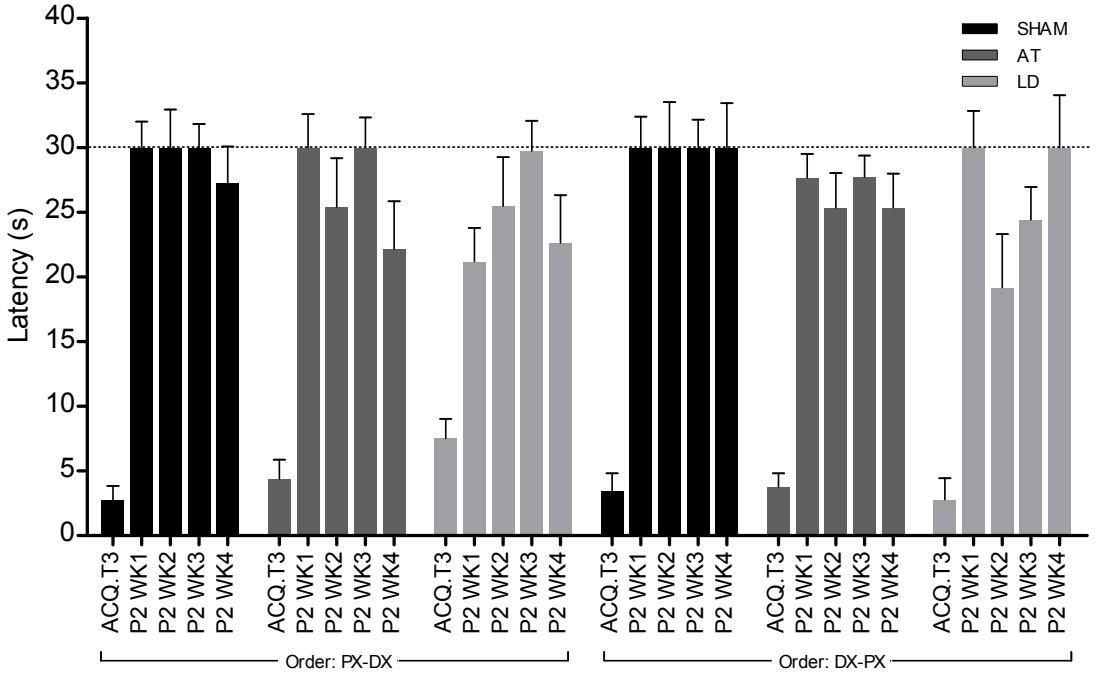

Figure 3.29. Proximal task, Probe 2: Allocentric measure. Comparison of probe trials (WK1 – WK4; beacon removed, novel start, standard configuration intact) versus ACQ.T3 (beacon and standard configuration intact). Compared to acquisition, latency increased for all three lesion groups. No effect of order or weeks was seen. There was a significant difference between the lesion groups with the poorest performance seen in the sham group, and the best performance seen in the LD lesion group. The AT lesion group showed intermediate performance compared to the sham and LD lesion groups. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.3.2. Distal Task (Probe 2 – Allocentric measure)

As per the proximal task, allocentric navigation strategies were assessed by removing the beacon and releasing the rats from a novel start point relative to the standard configuration (Figure 3.30). Again, it was expected that AT lesioned rats would show the greatest impairment and LD lesioned rats would show an intermediate impairment compared to sham rats. It was expected that sham lesioned rats would exhibit slightly poorer performance compared to acquisition, as the task was considered more difficult.

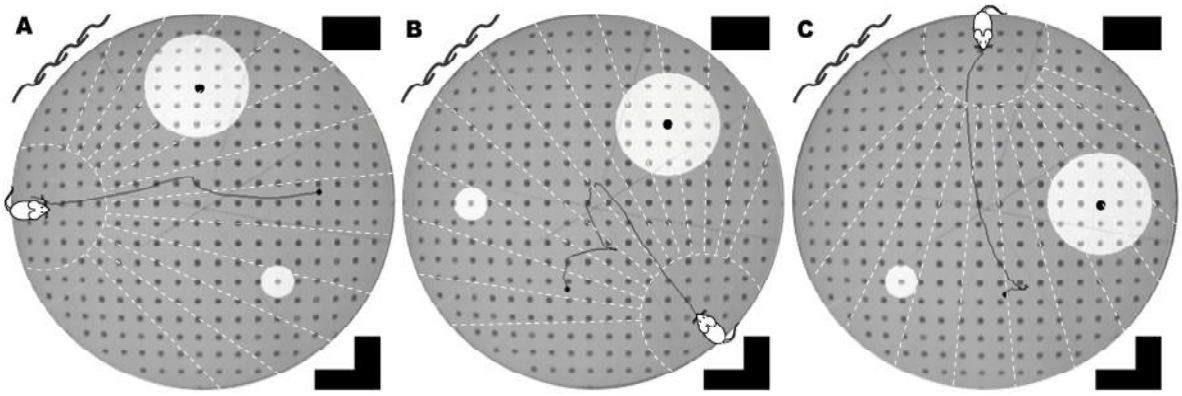

Figure 3.30. Distal task, Probe 2: Allocentric measure. Examples of run paths during probe trials. A) Sham (standard score = 4; modified score = 4); B) AT (standard score = 4; modified score = 13); C) LD (standard score = 7; modified score = 10). The distal cues represent the curtain and salient room cues, but are not drawn to scale.

Figure 3.31A shows the performance of rats in the distal task across four weeks of probe trials (P2a WK1 – P2a WK4), when released from a novel start point compared to acquisition (ACQ.T3). A marked increase in deviation scores was observed across the four probe trials compared to acquisition (Task effect, $F_{(4,164)} = 15.04$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 123.83$, $p < 0.001$), but not from each other. This suggests that when released from a novel start point, rats failed to use the spatial relationship of the distal cues to guide navigation. However, the paths shown in Figure 3.30 do not show the same degree of deviation from the reward location as the proximal cue condition (Figure 3.26). The initial heading trajectories are less obviously governed by the learned egocentric trajectory, indicating that distal cues facilitate slightly more accurate spatial navigation.

Figure 3.31B shows the probe trial data from Figure 3.31A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the four probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.2$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

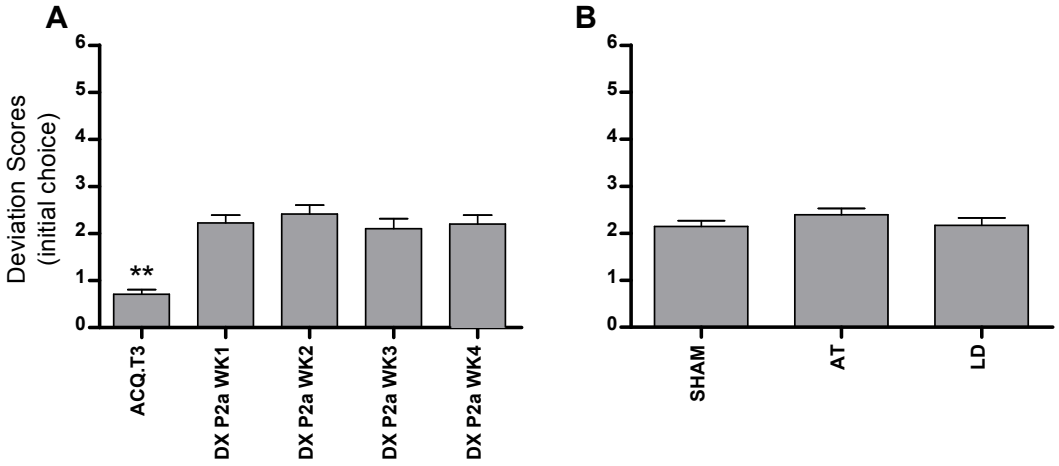

Figure 3.31. Distal task, Probe 2: Allocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of $p < 0.001$.

Figure 3.32 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$) and across weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction ($F < 1.0$).

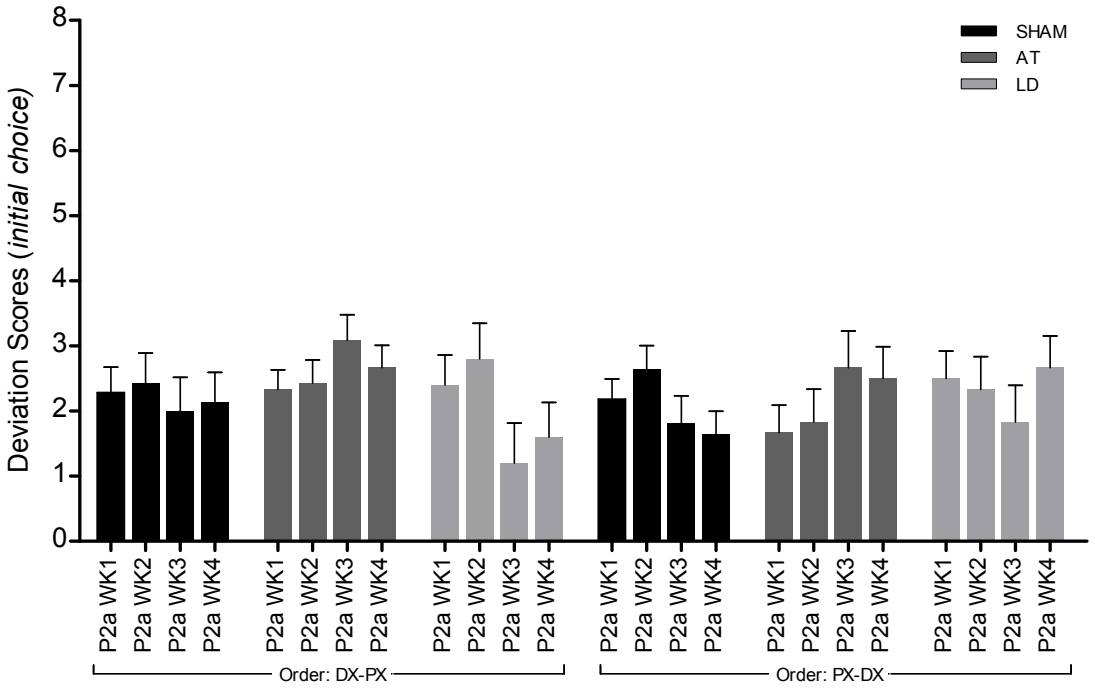

Figure 3.32. Distal task, Probe 2: Allocentric measure. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

Figure 3.33 shows the latency for each lesion group to locate the food reward in probe trials compared to acquisition (ACQ.T3) across task order conditions and weeks. The increase in latency across the probe trials compared to acquisition trials (ACQ.T3) was marked, with many trials nearing the maximum (30 second) trial duration (Task effect, $F_{(4,164)} = 32.33$, $p < 0.001$). A planned comparison confirmed that all probe trials differed significantly from acquisition ($F_{(1,41)} = 250.49$, $p < 0.001$), but not from each other. The high latency scores reflect that the rats often failed to locate the food reward area when released from a novel start. Comparing only the probe trials, there were no effects of Lesion or Order (Fs < 1.6), nor was there a Lesion x Order, Week x Lesion or Week x Order interaction ($F < 1.5$).

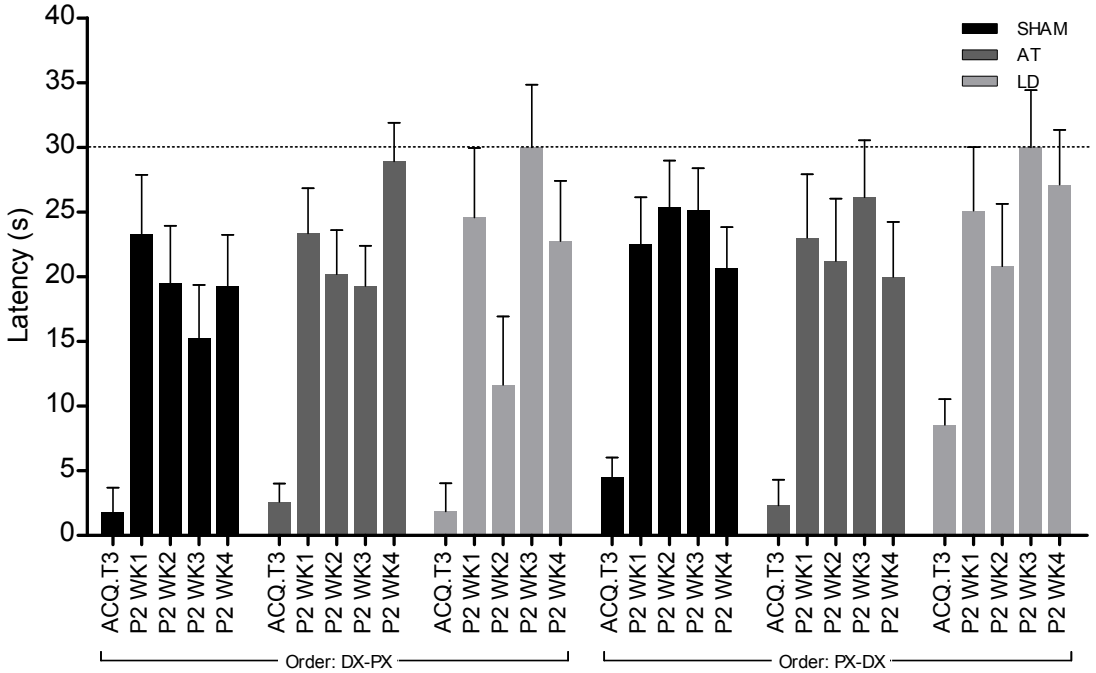

Figure 3.33. Distal task, Probe 2: Allocentric measure. Comparison of probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) versus ACQ.T3 (beacon and standard configuration intact). Mean latency (±SEM) to locate the food reward across task order and weeks for each lesion group. Compared to acquisition, latency increased for all three lesion groups. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.4. Probe 2: Egocentric Scores

3.3.4.1. Proximal Task (Probe 2 – Egocentric measure)

If rats were unable to show good allocentric performance, this could be due to the use of an egocentric strategy. As such, data from the same probe were transposed to obtain an egocentric score (refer back to Figure 3.3, page 43).

Figure 3.34A shows similar scores to Figure 3.27. This is likely due to the standard scoring method used in this analysis, where an allocentric mean score of '3' would transpose into an egocentric mean score of '4'. A marked increase in deviation scores was observed across the four probe trails compared to ACQ.T3 (Task effect, $F_{(4,164)} = 6.67$, $p < 0.001$). A planned comparison confirmed that the four probe trials differed from ACQ.T3 ($F_{(1, 41)} = 16.23$, $p < 0.001$), but not from each other. Figure 3.34B shows the probe trial data from Figure 3.34A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham

group (Lesion effect, $F < 1.0$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

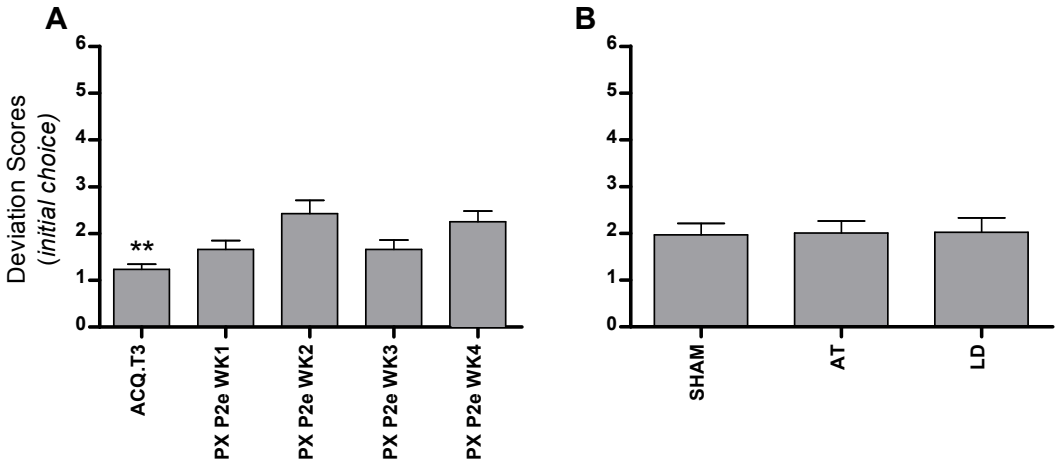

Figure 3.34. Proximal task, Probe 2: Egocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of p < 0.001.

Figure 3.35 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$). Differences were seen between weeks (Week effect, $F_{(3,123)} = 4.20$, $p < 0.001$), but these were not linear, which argues against a learning effect. There was no Lesion x Order interaction, but there was a Week x Lesion ($F_{(6,123)} = 2.10$, $p = 0.06$) and a Week x Lesion x Order interaction ($F_{(6,123)} = 3.54$, $p < 0.01$).

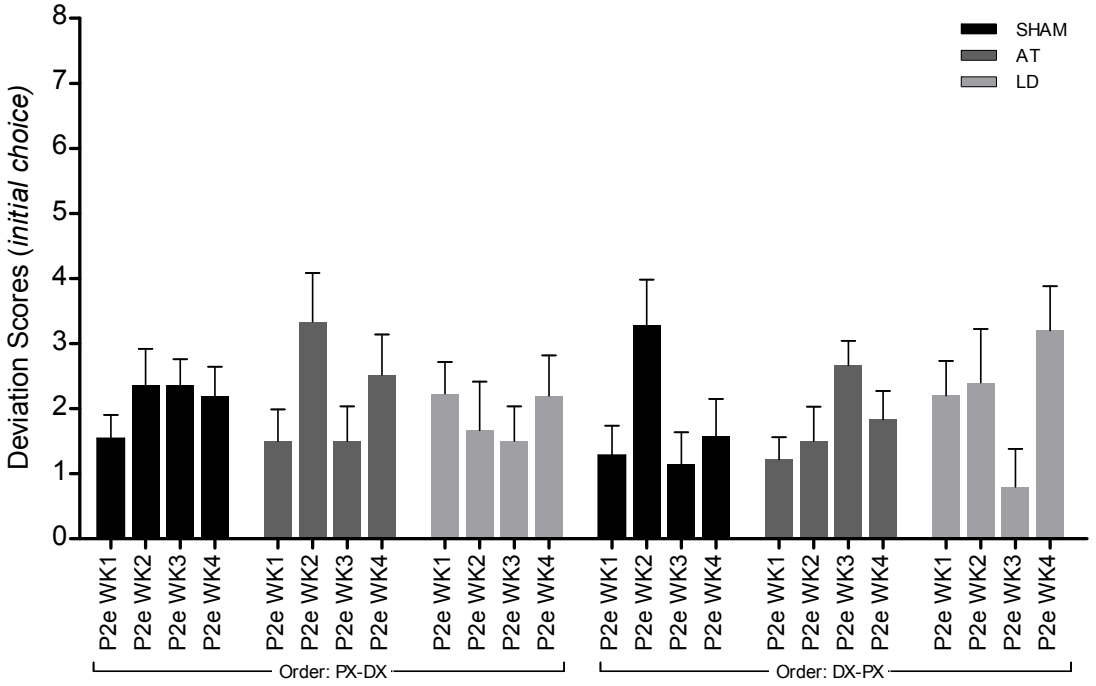

Figure 3.35. Proximal task, Probe 2: Egocentric measure. Mean deviation (±SEM) across task order and weeks for each lesion group. No effects of order or lesion were seen, but there was an effect of week. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.4.2. Distal Task (Probe 2 – Egocentric measure)

As per the proximal task, data were transposed to obtain an egocentric score. Figure 3.36A shows almost identical scores to Figure 3.31. As discussed previously, this is likely due to the standard scoring method used in this analysis. A marked increase in deviation scores was observed across the four probe trails compared to ACQ.T3 (Task effect, $F_{(4,164)} = 21.92$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials differed from ACQ.T3 ($F_{(1, 41)} = 154.90$, $p < 0.001$), but not from each other. Figure 3.36B shows the probe trial data from Figure 3.36A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.4$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

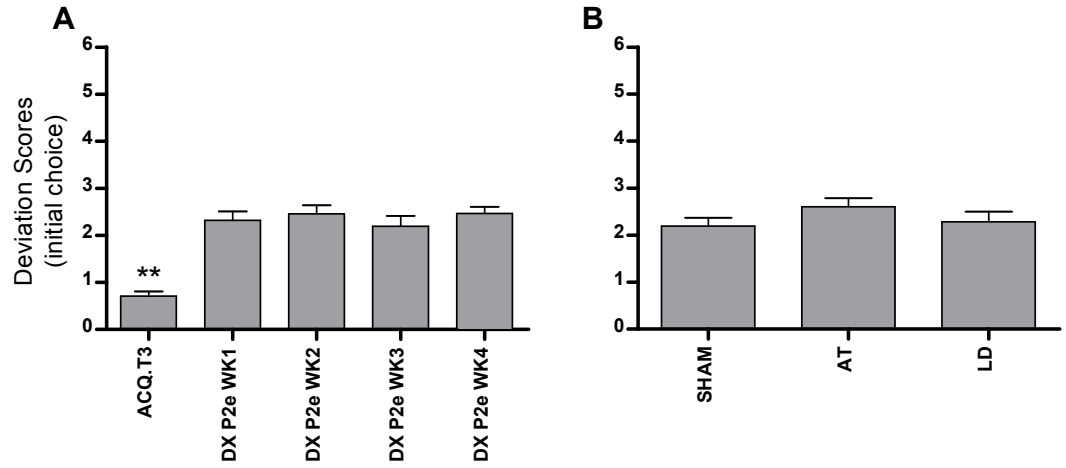

Figure 3.36. Distal task, Probe 2: Egocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of p < 0.001.

Figure 3.37 shows how accurately each lesion group navigated toward the food reward, but now across task order conditions (PX-DX, DX-PX) for probe trials. Performance was similar for both task Order conditions ($F < 1.0$) and across Weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction ($F < 2.0$).

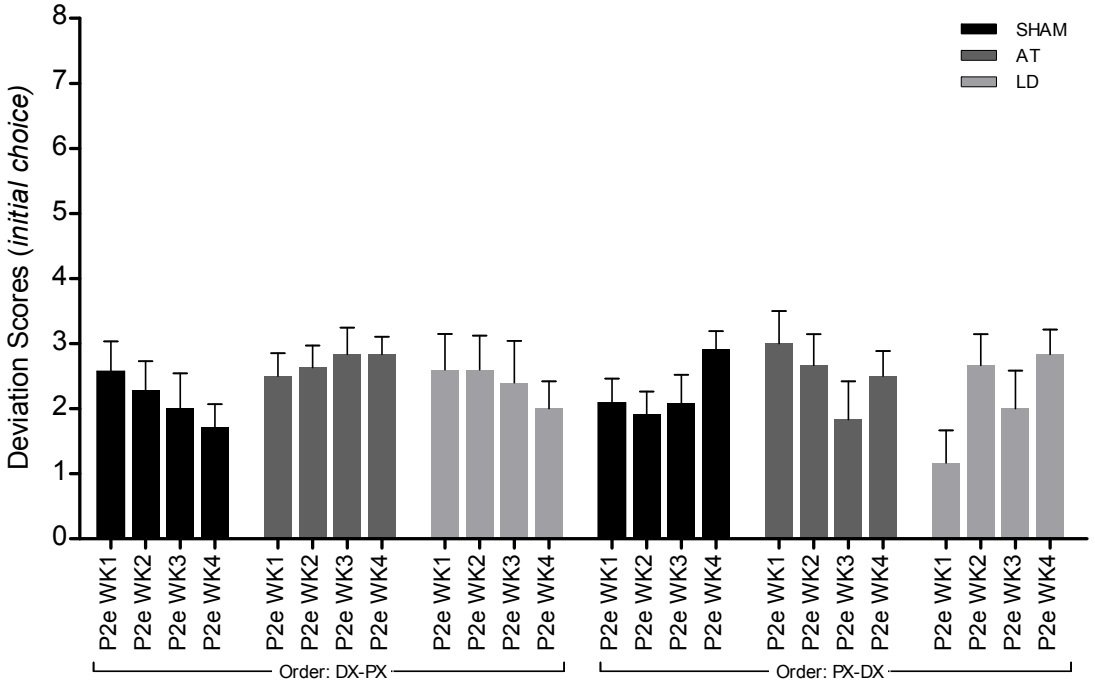

Figure 3.37. Distal task, Probe 2: Egocentric measure. Mean deviation (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.5. Probe 2: Modified Scoring System

3.3.5.1. Proximal Task (Probe 2 – Allocentric [Modified Scoring System])

The higher deviation scores seen in Figure 3.38A (compared to Figure 3.27A) were due to the greater number of zones being utilised. Deviation scores were significantly higher across the four probe trials compared to ACQ.T3 (Task effect, $F_{(4,164)} = 43.83$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 579.10$, $p < 0.001$), but did not differ from each other. This indicates that when released from a novel start point, while proximal cues were available, accurate navigation to the reward location was severely impaired. Figure 3.38B shows the probe trial data from Figure 3.38A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.5$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

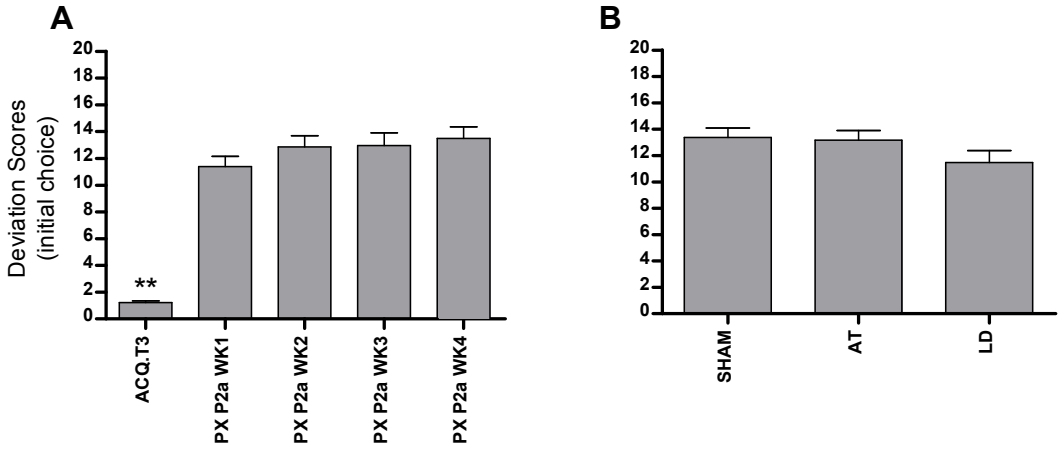

Figure 3.38. Proximal task, Probe 2: Modified scoring system, allocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of $p < 0.001$.

Figure 3.39 shows how accurately each lesion group navigated towards the food rewards, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$) and across Weeks ($F < 1.2$). There was no Lesion x Order, Week x Lesion or Week x Order interaction ($F < 1.3$).

Thus, increasing the sensitivity of the deviation measure did not alter the main findings when proximal cues were available to guide navigation. However, the merits of using the modified scoring system will be discussed further in Section 3.3.6.

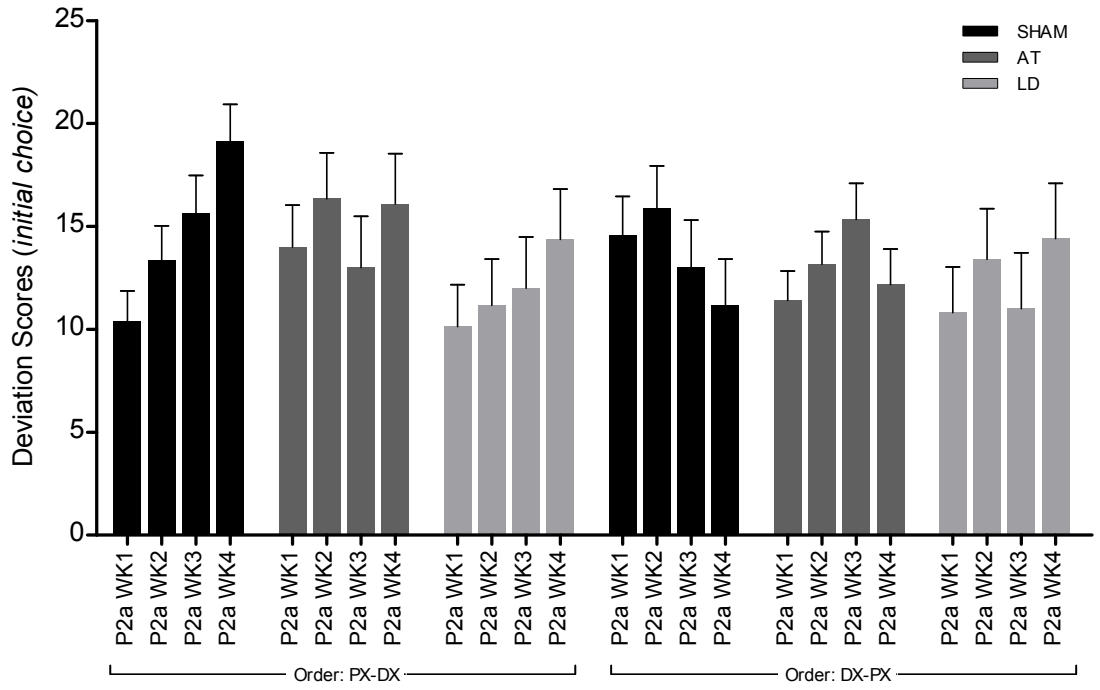

Figure 3.39. Proximal task, Probe 2: Modified scoring system, allocentric measure. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.5.2. Distal Task (Probe 2 – Allocentric [Modified Scoring System])

As with the proximal task, the higher deviation scores seen in Figure 3.40A (compared to Figure 3.31A) were due to the greater number of zones being utilised. Deviation scores were significantly higher across the four weeks of probe trials compared to ACQ.T3 (Task effect, $F_{(4,164)} = 9.40$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 109.66$, $p < 0.001$), but did not differ from each other. This indicates that when released from a novel start point, while distal cues were available, accurate navigation to the reward location was impaired. Figure 3.40B shows the probe trial data from Figure 3.40A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that across the four probe trials, the AT and LD lesion groups performed similarly to the sham groups (Lesion effect, $F < 1.5$).

Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

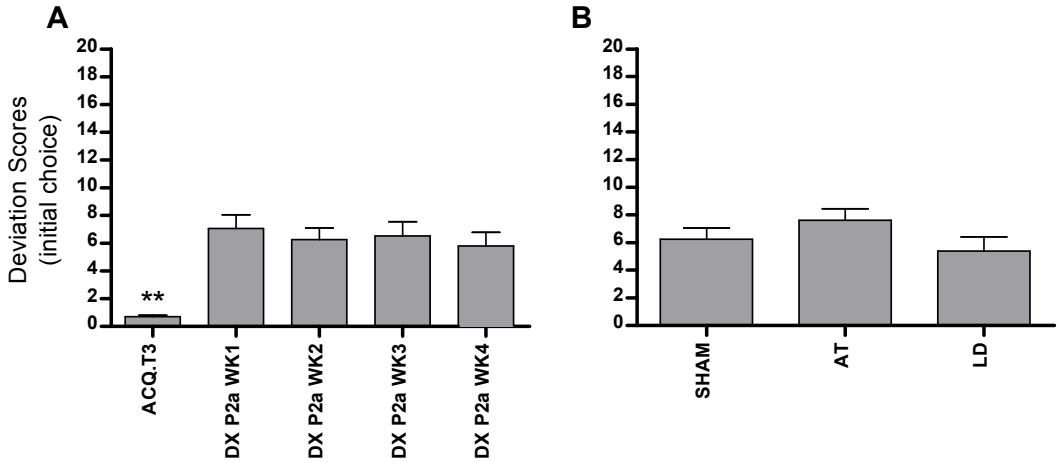

Figure 3.40. Distal task, Probe 2: Modified scoring system, allocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of p < 0.001.

Figure 3.41 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.5$) and across Weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 1.5). As with the proximal task, increasing the sensitivity of the deviation measure did not alter the main findings when distal cues were available to guide navigation. However, the merits of using the modified scoring system will be discussed further in Section 3.3.6.

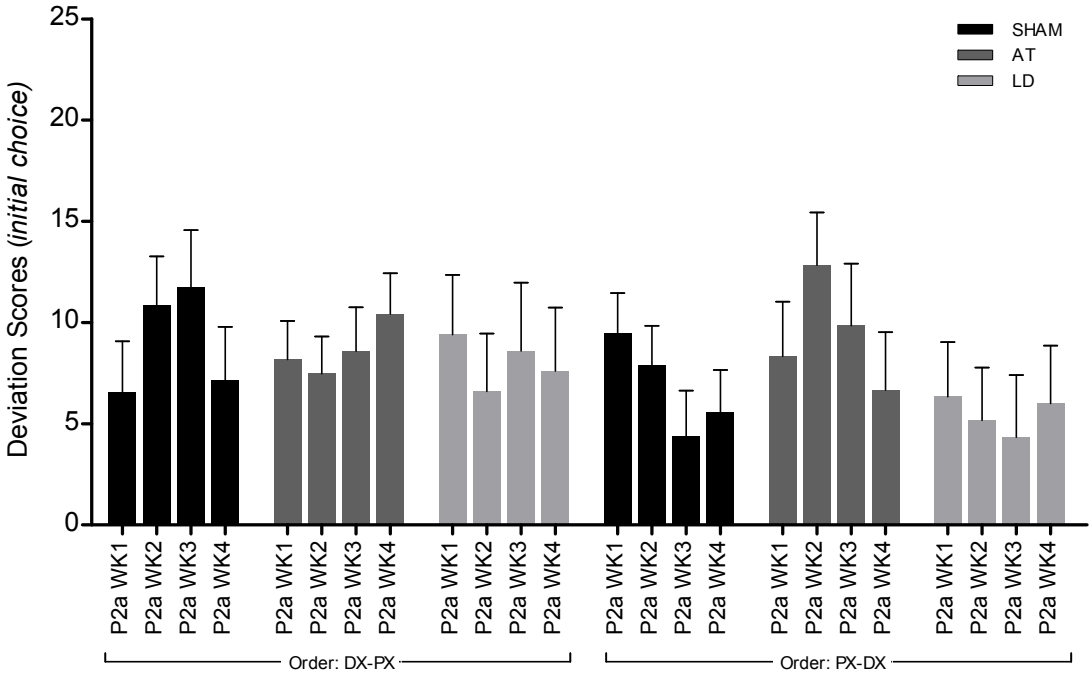

Figure 3.41. Distal task, Probe 2: Modified scoring system, allocentric measure. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.5.3. Proximal Task (Probe 2 – Egocentric [Modified Scoring System])

The lower deviation scores seen in Figure 3.42A (Figure 3.34A) emerged because rats ran more directly to the expected food reward location (egocentric navigation) compared to the actual food reward location (allocentric navigation). Deviation scores were significantly higher across the four probe trials compared to ACQ.T3 (Task effect, $F_{(4,164)} = 11.70$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 75.25$, $p < 0.001$), but not from each other. Figure 3.42B shows the probe trial data from Figure 3.42A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.9$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

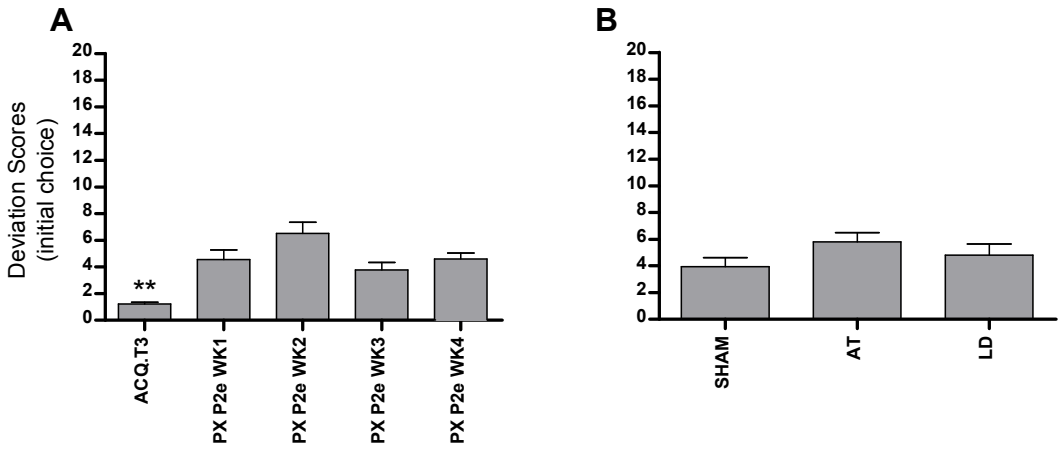

Figure 3.42. Proximal task, Probe 2: Modified scoring system, egocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of p < 0.001.

Figure 3.43 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$). Differences were seen between weeks (Week effect, $F_{(3,123)} = 3.70$, $p < 0.05$) but these were not linear, which argues against a learning effect. There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 2.1). Increasing the sensitivity of the deviation measure did not alter the main findings when proximal cues were available to guide navigation. However, the merits of using the modified scoring system will be discussed further in Section 3.3.6.

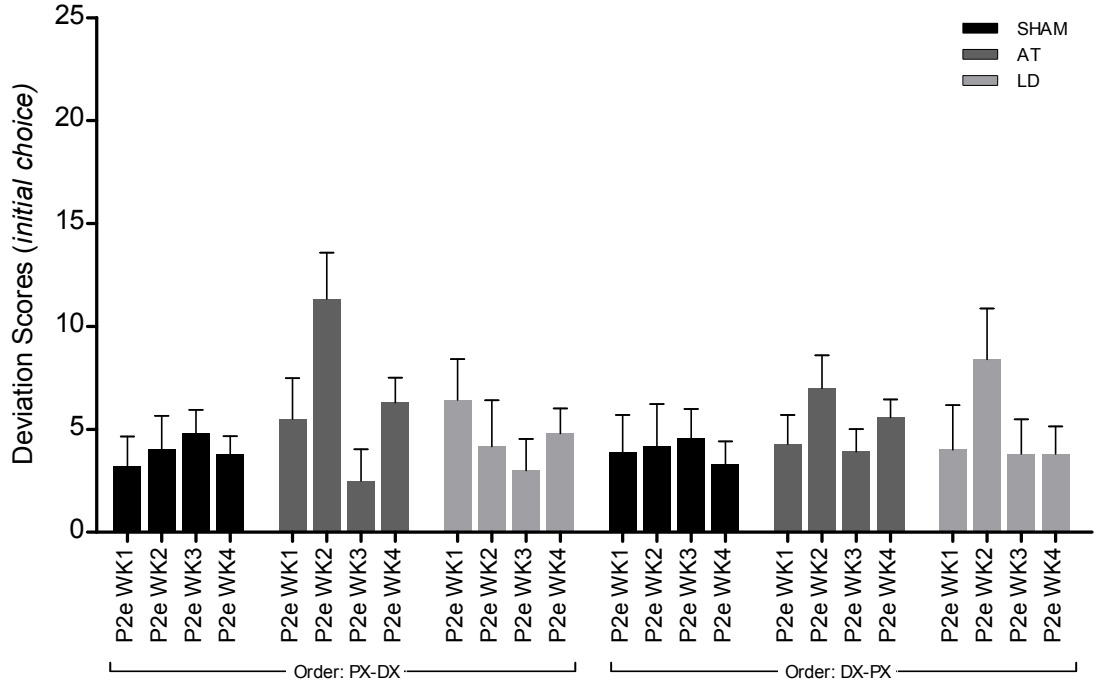

Figure 3.43. Proximal task, Probe 2: Modified scoring system, egocentric measure. Mean deviation (±SEM) across task order and weeks for each lesion group. No effects of order or lesion were seen, but there was an effect of week. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.5.4. Distal Task (Probe 2 – Egocentric [Modified Scoring System])

As with the proximal task, the lower deviation scores seen in Figure 3.44A (compared to Figure 3.36A) emerged because rats ran more directly to the expected food reward location (egocentric navigation) compared to the actual food reward location (allocentric navigation). Deviation scores were significantly higher across the four probe trials compared to ACQ.T3 (Task effect, $F_{(4,164)} = 20.24$, $p < 0.001$). A planned comparison of the Task effect confirmed that the four probe trials were significantly different from ACQ.T3 ($F_{(1,41)} = 212.01$, $p < 0.001$), but did not differ from each other. Figure 3.44B shows the probe trial data from Figure 3.44A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.0$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

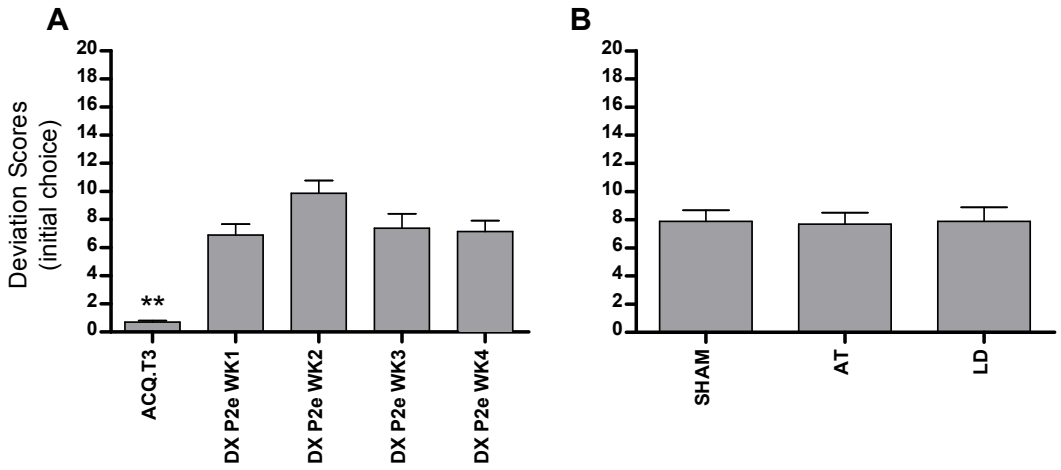

Figure 3.44. Distal task, Probe 2: Modified scoring system, egocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon removed, novel start point, standard configuration intact) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of p < 0.001.

Figure 3.45 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 3.1$). Differences were seen between weeks (Week effect, $F_{(3,123)} = 2.75$, $p < 0.05$) but these were not linear, which argues against a learning effect. There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 2.4). Increasing the sensitivity of the deviation measure did not alter the main findings when distal cues were available to guide navigation. However, the merits of using the modified scoring system will be discussed further in Section 3.3.6.

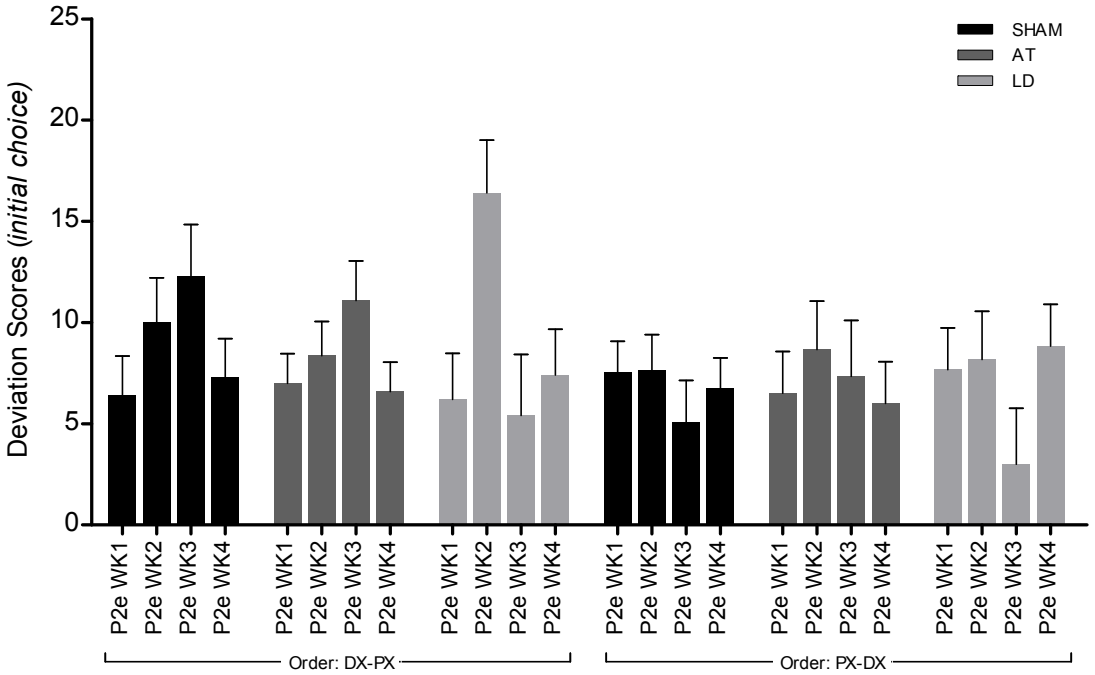

Figure 3.45. Distal task, Probe 2: Modified scoring system, egocentric measure. Mean deviation (±SEM) across task order and weeks for each lesion group. No effects of order or lesion were seen, but there was an effect of week. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.6. Probe 2: Justification for Two Scoring Systems

Although increasing the sensitivity of the deviation measure did not alter the main findings across the two task conditions, as shown by the increase in deviation scores across the four probe trials compared to acquisition, and the absence of lesion effects, use of the modified scoring system now exhibited differences between allocentric and egocentric navigation strategies.

3.3.6.1. Proximal Task (Probe 2 – Standard versus Modified Scoring System)

Figure 3.46 shows the comparative differences between allocentric and egocentric deviation scores using the *standard* scoring system when proximal cues were available to guide navigation from a novel start point. There was a small, but significant difference between the two tasks (Task effect, $F_{(1,41)} = 12.40$, $p < 0.001$).

Figure 3.47 shows the comparative differences between allocentric and egocentric deviation scores using the *modified* scoring system when proximal cues were available to guide navigation from a novel start point. Now, the allocentric scores were much higher

than egocentric scores (Task effect, $F_{(1,41)} = 161.79$, $p < 0.001$). The exaggerated difference implies that using proximal cues to guide navigation from a novel start was relatively difficult, and therefore, an egocentric strategy was used preferentially. Despite using an egocentric trajectory in this task, performance was still much poorer than in acquisition trials.

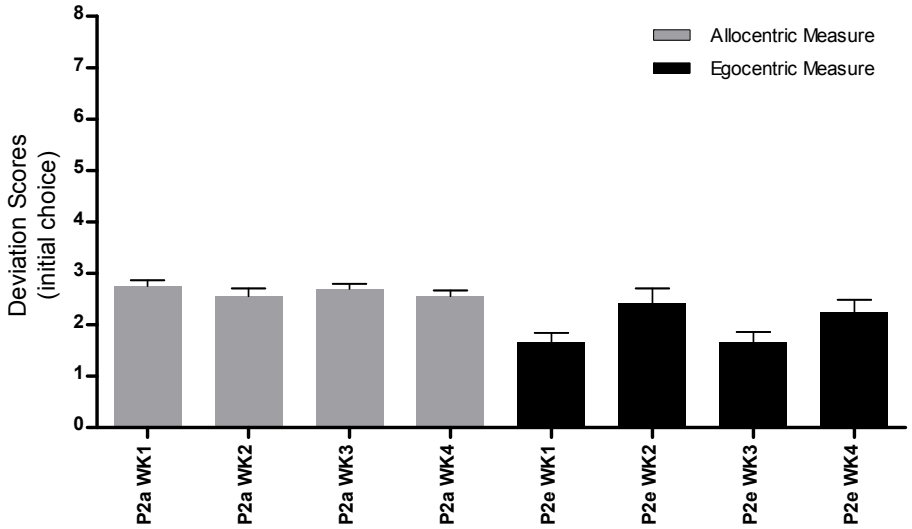

Figure 3.46. Proximal task, Probe 2: Standard scoring system. The mean deviation scores (±SEM) to the food reward (allocentric) and the expected food reward (egocentric) location were calculated separately. The deviation scores to the food reward were significantly higher than the deviation scores to the expected food location suggesting the use of an egocentric strategy.

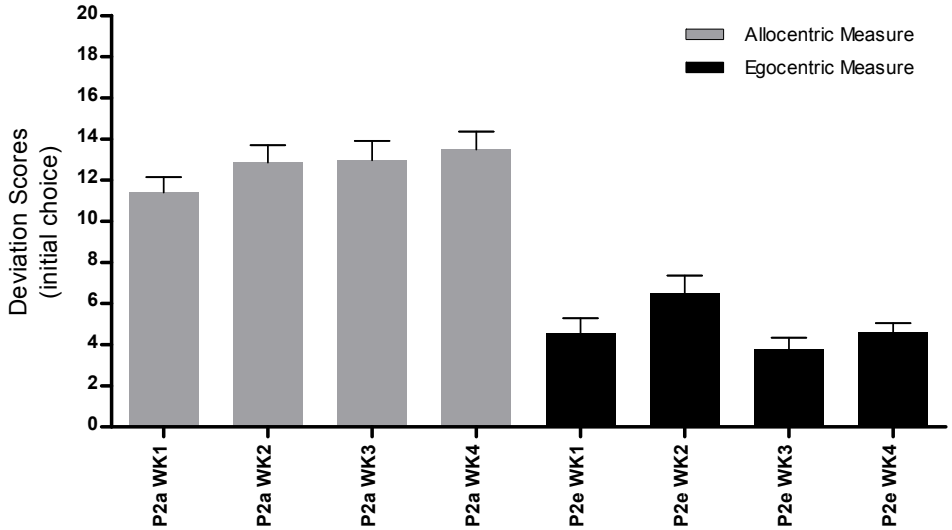

Figure 3.47. Proximal task, Probe 2: Modified scoring system. The mean deviation scores (±SEM) to the food reward (allocentric) and the expected food reward (egocentric) location were calculated separately. The deviation scores to the food reward were significantly higher than the deviation scores to the expected food location suggesting the use of an egocentric strategy.

3.3.6.2. Distal Task (Probe 2 – Standard versus Modified Scoring System)

Figure 3.47 shows the comparative differences between allocentric and egocentric deviation scores using the *standard* scoring system when distal cues were available to guide navigation from a novel start point. Unlike the proximal task, there were no significant differences between the two tasks (Task effect, $F < 1.1$).

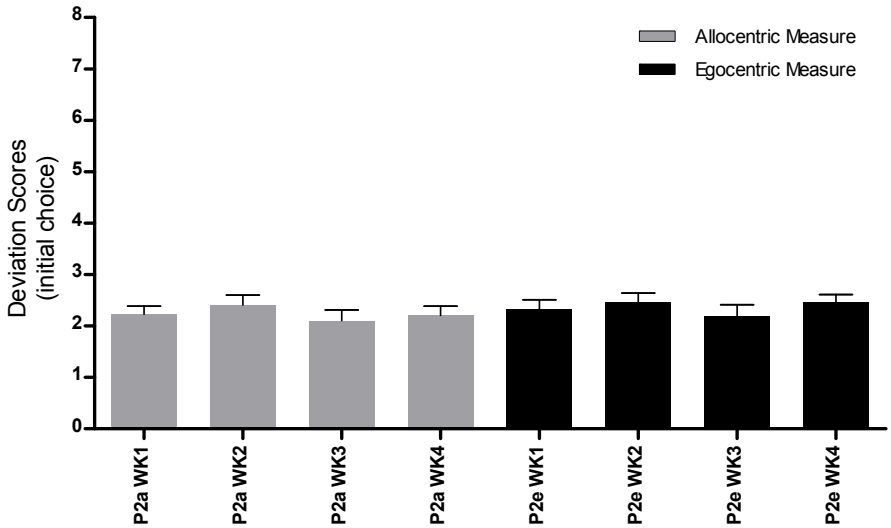

Figure 3.48. Distal task, Probe 2: Standard scoring system. The mean deviation scores (±SEM) to the food reward (allocentric) and the expected food reward (egocentric) location were calculated separately. The deviation scores to the food reward were similar to the deviation scores to the expected food location suggesting that neither strategy was preferentially used.

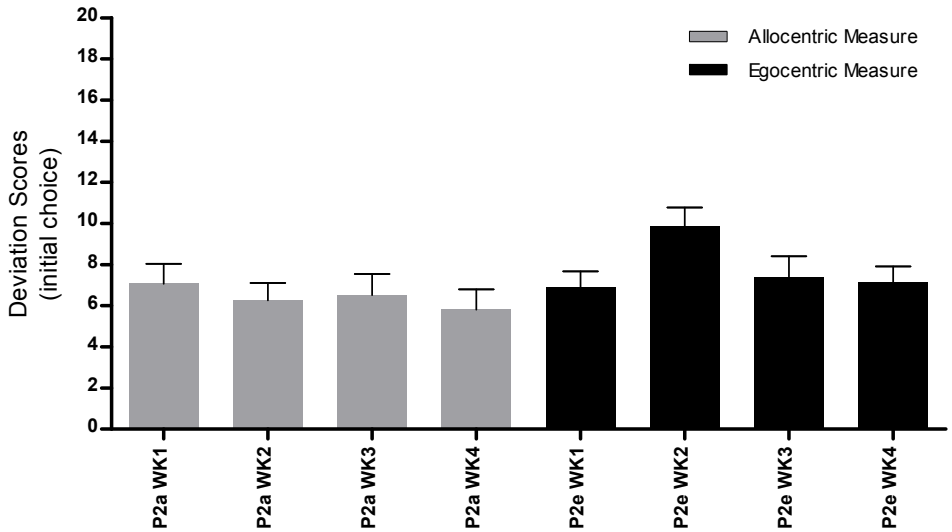

Figure 3.49. Distal task, Probe 2: Modified scoring system. The mean deviation scores (±SEM) to the food reward and the expected food reward location were calculated separately. The deviation scores to the food reward were similar to the deviation scores to the expected food location suggesting that neither strategy was preferentially used.

Figure 3.49 shows the comparative differences between the allocentric and egocentric deviation scores using the *modified* scoring system when distal cues were available to guide navigation from a novel start point. Similar to that of the proximal task, the scores were now slightly higher in the egocentric measure (Task effect, $F_{(1,41)} = 4.96$, $p < 0.05$). The slight increase in egocentric scores suggests that using distal cues to help navigate from a novel start point did not elicit a strong strategy preference.

3.3.7. Probe 3: Egocentric Probe

Table 3.4. Probe 3 Parameters.

Beacon	Start Point	Visual Cues
Removed	*Standard (no change)*	*Removed*

3.3.7.1. Proximal Task (Probe 3)

Probe 3 assessed whether rats were able to navigate using proprioceptive and vestibular cues instead of visual cues. All visual cues, including the beacon, were removed (Figure 3.50). In contrast to probe 2, this probe was expected to encourage egocentric strategies, so it was expected that AT lesioned rats would now no impairments, and LD lesioned rats would show the marked impairments compared to sham rats. It was expected that sham lesioned rats would exhibit similar performance compared to acquisition.

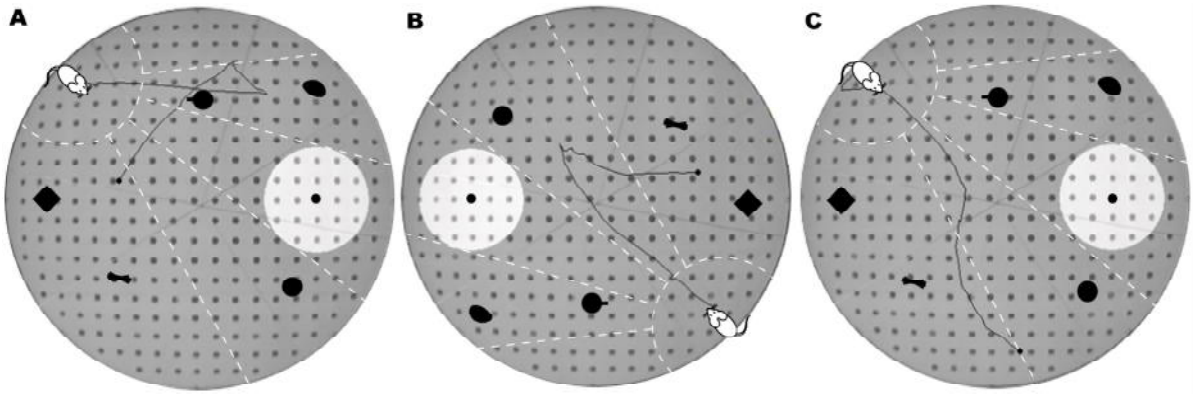

Figure 3.50. Proximal task, Probe 3: Egocentric navigation. Examples of run paths during probe trials. A) Sham (score = 4); B) AT (score = 4); C) LD (score = 5). During these probe trials, the cues were not present. They have been included for illustrative purposes only.

Figure 3.51A shows the performance of rats in the proximal task across four weeks of probe trials (P3 WK1 – P3 WK4) when all visual cues were removed compared to

acquisition (ACQ.T3). A marked increase in deviation scores was observed across the four probe trials compared to ACQ.T3 (Task effect, $F_{(4,164)} = 8.24$, $p < 0.001$). A planned comparison of the Task effect confirmed that all probe trials differed from ACQ.T3 ($F_{(1,41)} = 93.55$, $p < 0.001$), but not from each other. This suggests that rats' heading direction was influenced by visual cues and not solely proprioceptive and vestibular cues. This is supported by the running paths shown in Figure 3.50. There appears to be a short distance which was based on the learned trajectory, but this diminished quickly, resulting in higher deviation scores compared to probes when cues were available to facilitate navigation (i.e. Figures 3.17 and 3.26). Figure 3.51B shows the probe trial data from Figure 3.51A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.0$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

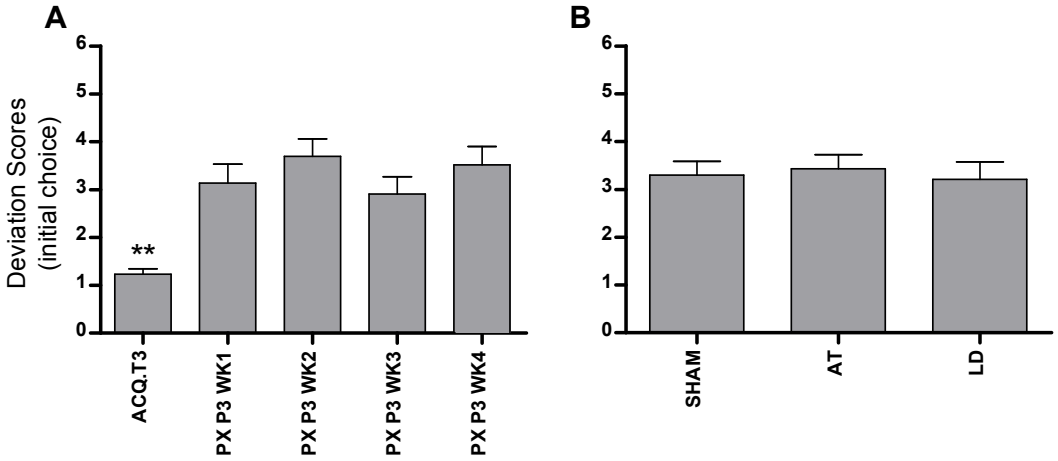

Figure 3.51. Proximal task, Probe 3: Egocentric navigation. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon and all visual cues removed) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of p < 0.001.

Figure 3.52 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials.

Performance was similar for both task Order conditions ($F < 1.0$) and across Weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 1.3).

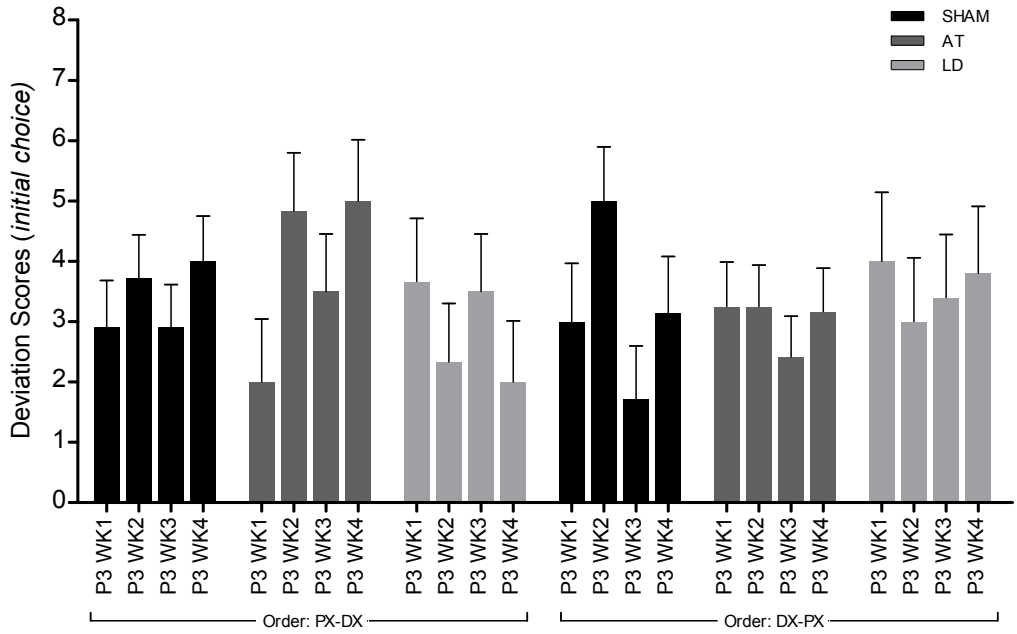

Figure 3.52. Proximal task, Probe 3: Egocentric navigation. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

Figure 3.53 shows the latency for each lesion group to locate the food reward in probe trials compared to acquisition (ACQ.T3) across task order conditions and weeks. There was a marked increase in latency in the probe trials compared to acquisition trials (ACQ.T3) (Task effect, $F_{(4,41)} = 20.26$, $p < 0.001$). A planned comparison confirmed that all probe trials differed significantly from acquisition ($F_{(1,41)} = 265.86$, $p < 0.001$), but not from each other. The high latency scores reflect that the rats failed to locate the food reward area when they had to rely solely on proprioceptive and vestibular cues to navigate. Comparing only the probe trials, there was some evidence of a difference between the lesion groups (Lesion effect, $F(2,41)=2.46$, $p = 0.09$). A planned comparison showed that the LD lesion group performed worse than the sham group ($t = 2.22$, $p < 0.05$), and the AT lesion group did not differ from either the sham or LD lesion groups. There was no effect of Order ($F < 1.0$), nor was there a Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 1.5).

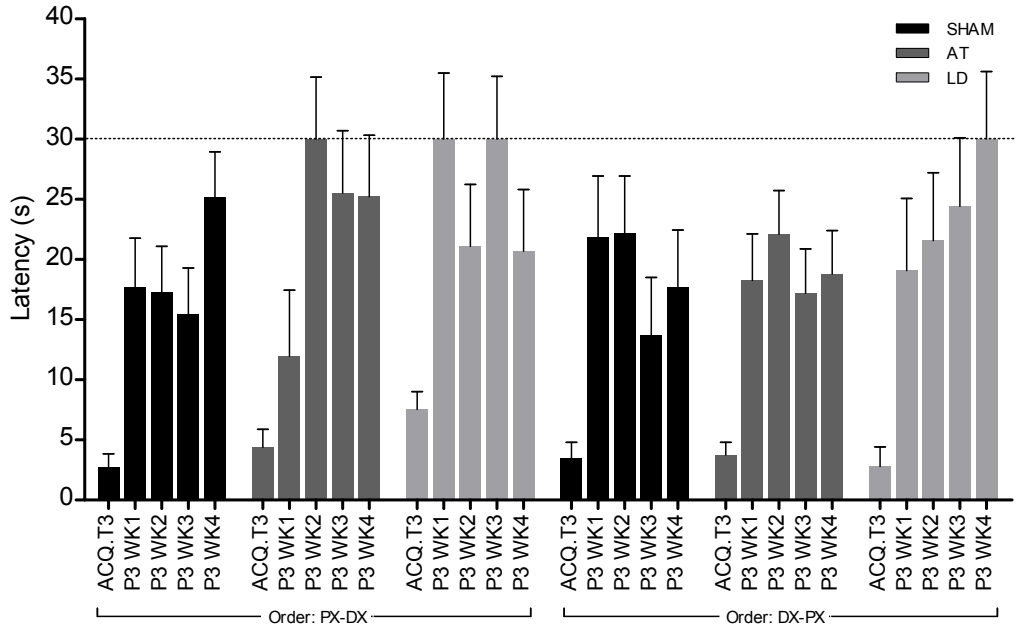

Figure 3.53. Proximal task, Probe 3: Egocentric navigation. Comparison of probe trials (WK1 – WK4; beacon removed with standard configuration intact) versus ACQ.T3 (beacon and standard configuration intact). Mean latency (±SEM) to locate the food reward across task order and weeks for each lesion group. Compared to acquisition, latency increased for all three lesion groups. No effects of order or week were seen. There was a significant difference between the lesion groups with the poorest performance seen in the LD lesion group, and the best performance seen in the sham group. The AT lesion group showed intermediate performance compared to the sham and LD lesion groups.

3.3.7.2. Distal Task (Probe 3)

As per the proximal task, Probe 3 assessed whether rats could navigate without any visual cues. All visual cues, including the beacon, were removed (Figure 3.54). Again, because this probe may emphasise egocentric strategies, it was expected that AT lesioned rats would show no impairments, but the LD would show marked impairments compared to the sham rats. It was expected that sham lesioned rats would exhibit similar performance compared to acquisition.

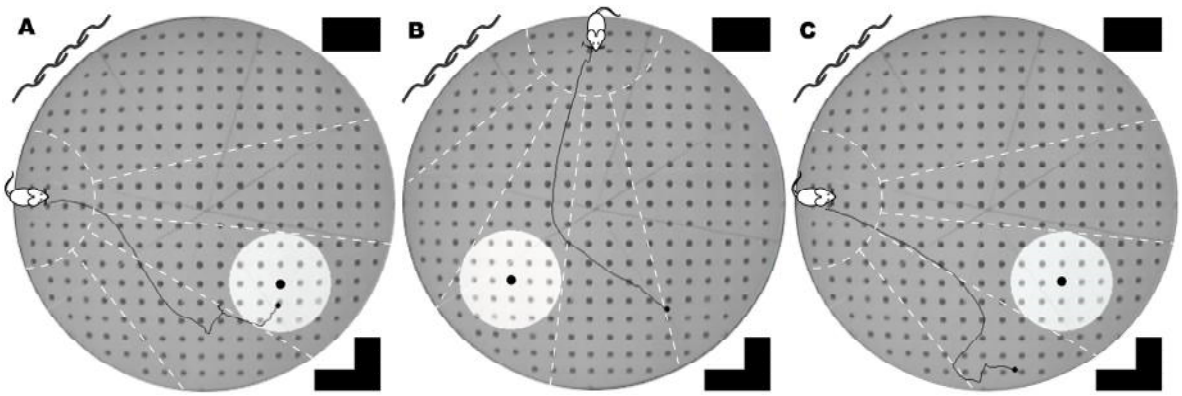

Figure 3.54. Distal task, Probe 3: Egocentric navigation. Examples of run paths during probe trials. A) Sham (score = 2); B) AT (score = 4); C) LD (score = 4).

Figure 3.55A shows the performance of rats in the distal task across four weeks of probe trials (P3 WK1 – P3 WK4), when all visual cues were removed compared to acquisition (ACQ.T3). A marked increase in deviation scores was observed across the four probe trials compared to ACQ.T3 (Task effect $F_{(4,164)} = 8.23$, $p < 0.001$). A planned comparison of the Task effect confirmed that all probe trials differed from ACQ.T3 ($F_{(1, 41)} = 66.78$, $p < 0.001$), but not from each other. This suggests that rats' heading direction was influenced by visual cues and not solely proprioceptive and vestibular cues. This is supported by the running paths shown in Figure 3.54. There appears to be a short distance which was based on the learned trajectory, but this diminished quickly, resulting in higher deviation scores compared to probes when cues were available to facilitate navigation (i.e. Figures 3.21 and 3.30). Figure 3.55B shows the probe trial data from Figure 3.55A (i.e. excluding ACQ.T3) broken down by lesion type. This shows that for the probe trials, the AT and LD lesion groups performed similarly to the sham group (Lesion effect, $F < 1.3$). Performance of the sham group was moderately disrupted, and may therefore explain the absence of lesion effects.

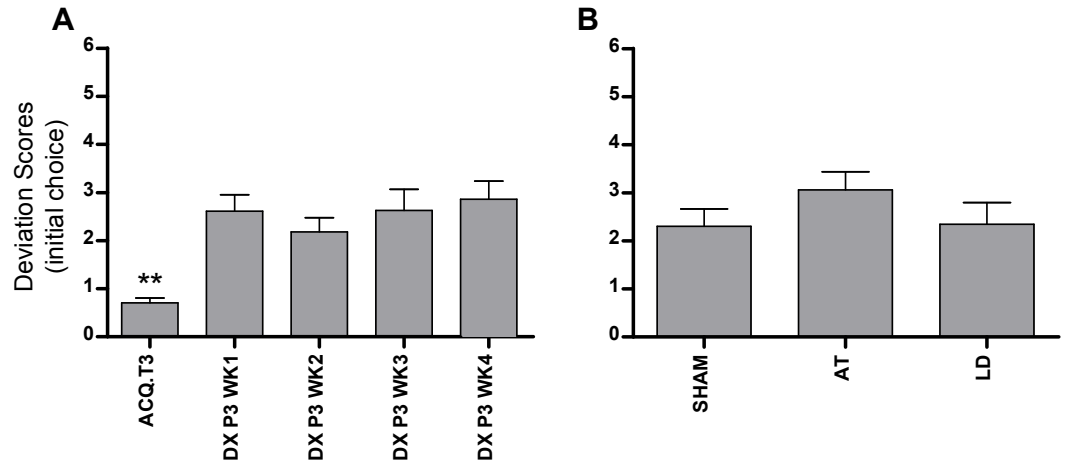

Figure 3.55. Distal task, Probe 3: Egocentric measure. A) There was a significant difference between the mean deviation scores (±SEM) of all probe trials (WK1 – WK4; beacon and all visual cues removed) compared to ACQ.T3 (beacon and standard configuration intact). B) No significant differences were seen when comparing the lesion groups across the four probe trials (WK1 – WK4). ** Indicates statistical significance of p < 0.001.

Figure 3.56 shows how accurately each lesion group navigated towards the food reward, but now across task order conditions (PX-DX, DX-PX) for the probe trials. Performance was similar for both task Order conditions ($F < 1.0$) and across Weeks ($F < 1.0$). There was no Lesion x Order, Week x Lesion or Week x Order interaction (Fs < 1.3).

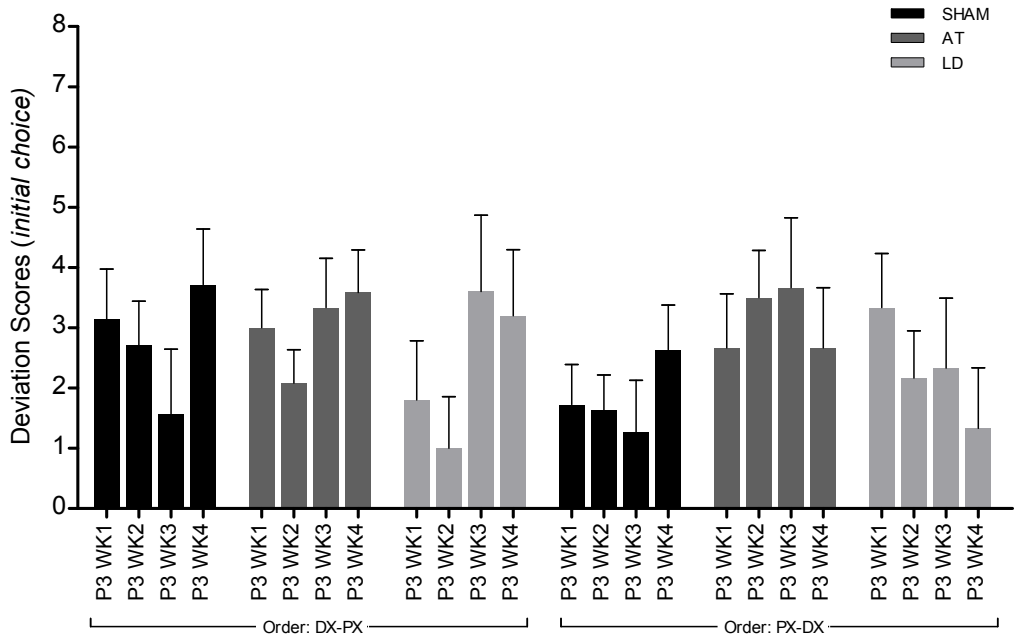

Figure 3.56. Distal task, Probe 3: Egocentric measure. Mean deviation scores (±SEM) across task order and weeks for each lesion group. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

Figure 3.57 shows the latency for each lesion group to locate the food reward in probe trials compared to acquisition (ACQ.T3) across task order conditions and weeks. There was a marked increase in latency in the probe trials compared to acquisition trials (ACQ.T3) (Task effect, $F_{(4,164)} = 17.08$, $p < 0.001$). A planned comparison confirmed that all probe trials differed significantly from acquisition ($F_{(1,41)} = 116.43$, $p < 0.001$), but not from each other. The high latency scores reflect that the rats failed to locate the food reward area when they had to rely solely on proprioceptive and vestibular cues to navigate. Comparing only probe trials, there were no effects of Lesion or Order (Fs < 1.0), nor was there a Lesion x Order or Week x Lesion interaction (Fs < 1.0). The Week x Order interaction just failed to reach significance ($F_{(3,123)} = 2.60$, $p = 0.06$), but no clear pattern is evident from Figure 3.57.

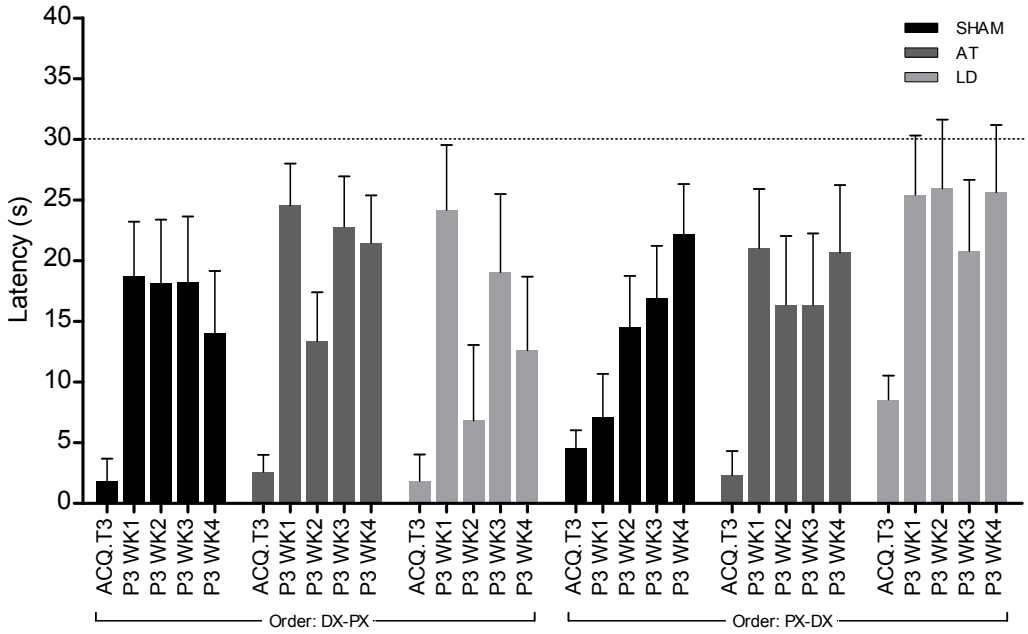

Figure 3.57. Distal task, Probe 3: Egocentric measure. Comparison of probe trials (WK1 – WK4; beacon removed with standard configuration intact) versus ACQ.T3 (beacon and standard configuration intact). Mean latency (±SEM) to locate the food reward across task order and weeks for each lesion group. Compared to acquisition, latency increased for all three lesion groups. No effects of order, week or lesion were seen. PX-DX: order of testing, proximal then distal; DX-PX: distal then proximal.

3.3.8. Comparison of Probes: Proximal versus Distal Cues to Guide Navigation

To assess how the proximity of visual cues affected navigation, the three probe manipulations were designed to be identical for both visual cue conditions, allowing direct comparisons to be made. Acquisition data have been excluded from the following analyses to directly compare probes trials across weeks. The data have also been collapsed across order and lesions to provide an overview of the navigation strategy differences when proximal and distal cues were available to guide navigation. The following figures show the difference in deviation scores and latency between the proximal and distal tasks for probes 1, 2 and 3 when egocentric or allocentric strategies were required to find a specific place.

Figure 3.58 illustrates that the rats deviated from the reward location in both visual cue conditions. However, overall performance was poorer when proximal cues facilitated navigation compared to distal cues (Task effect, $F_{(1,46)} = 70.56$, $p < 0.001$). Navigation accuracy also varied depending on the probe manipulations (Probe effect, $F_{(2,92)} = 10.65$, $p < 0.001$; Task x Probe interaction, $F_{(2,92)} = 15.45$, $p < 0.001$). Use of the standard scoring system for analysing probe 2 resulted in a narrow score range, and this is likely to have contributed to the Task x Probe interaction. No improvement in navigation accuracy was observed over the four probe weeks (Week effect, Task x Week, Task x Probe x Week interaction (Fs < 1.3).

When only the beacon was removed from the cue matrix (probe 1), rats showed higher deviation scores in their initial heading direction choice when proximal cues were still available to guide navigation. When distal cues were still available, the deviation scores were much lower than the when proximal cues were available which is indicative of more accurate navigation, but performance was still poorer than during acquisition, (Cue proximity effect for probe 1, $F_{(1,46)} = 85.50$, $p < 0.001$).

Performance in probe 3, when all visual cues were removed, remained stable in the proximal cue condition relative to probe 1 ($t_{(46)} = 0.32$, $p = 0.75$). However, in the distal cue condition, rats were less accurate when all the visual cues were removed, compared to only removing the beacon ($t_{(46)} = 4.78$, $p < 0.001$). The diminished performance in the distal cue condition, when no visual cues were available, highlights the influence distal cues have on navigation when they are available.

Compared to probe 1, when the beacon was removed and a novel start point was used to assess allocentric strategies (probe 2), deviation scores decreased slightly in the proximal cue condition ($t_{(46)} = 2.84$, $p < 0.05$), and increased in the distal cue condition ($t_{(46)} = 6.68$, $p < 0.001$), when the standard scoring system was used. As discussed in Section 3.3.6, and as seen in Figure 3.58, the standard scoring system produced a narrow score range, and despite them being significantly different ($t_{(46)} = 3.55$, $p < 0.001$), they did not fully reflect the true nature of the task. While the scores from the modified scoring system can't be directly compared to probe 1 because of the use of additional scoring zones, it is clear that the modified deviation scores distinguish the effects of cue proximity on navigation accuracy (Figure 3.58). The high deviation scores in the proximal cue condition suggest that the rats initially headed in the wrong direction, and continued to search for the reward location without deviating from the incorrect region.

Figure 3.59 illustrates that rats were slower to find the specific reward location when guided by proximal cues (Task effect, $F_{(1,46)} = 43.14$, $p < 0.001$). Latency was also affected by the different probe manipulations (Probe effect, $F_{(2,92)} = 87.98$, $p < 0.001$; Task x Probe interaction, $F_{(2,92)} = 2.63$, $p = 0.07$). No improvement in navigation accuracy was observed over the four probe weeks (Week effect, Task x Week or a Task x Probe x Week interaction, Fs < 1.6).

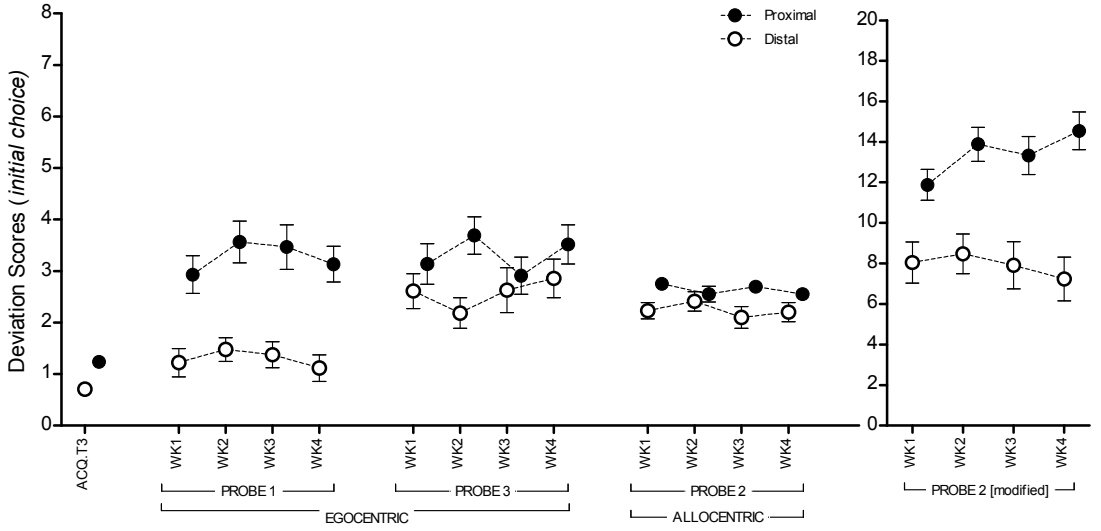

Figure 3.58. Proximal versus Distal tasks. Comparison of mean deviation scores (±SEM) across probes and weeks, categorized as egocentric and allocentric scores. The modified scores for probe 2 are included for clarity. Performance was consistently poorer when proximal cues guided navigation compared to distal cues. Error bars are not shown if the SEM is too small to be depicted.

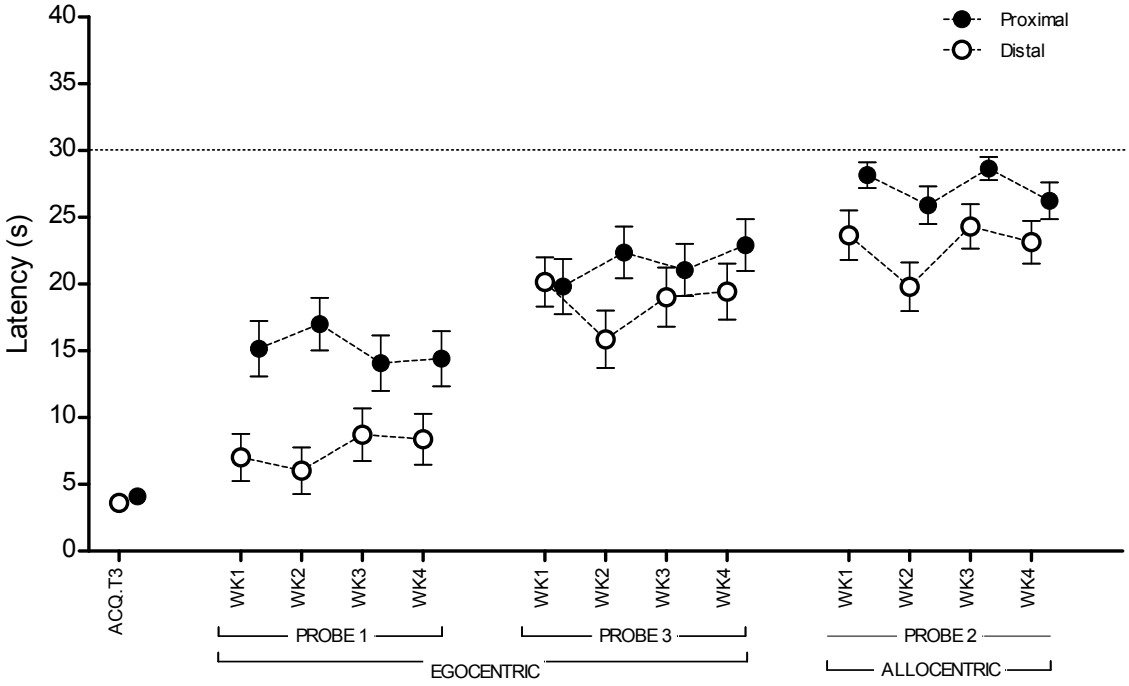

Figure 3.59. Proximal versus Distal tasks. Comparison of mean latency scores (±SEM) across probes and weeks, categorized as egocentric and allocentric scores. The latency to locate the food reward was impaired when only the beacon was removed, moderately impaired when all visual cues were removed and considerably impaired when a novel start point was used. Performance was consistently poorer when proximal cues guided navigation compared to distal cues. Error bars are not shown if the SEM is too small to be depicted.

When only the beacon was removed from the cue matrix (probe 1), rats were able to find the reward location but were slower to do so when guided by proximal cues. When distal cues were still available it took rats less time to locate the reward location which is indicative of a more effective and efficient search strategy, but performance was still poorer than during acquisition (Cue proximity effect for probe 1, $F_{(1,46)} = 26.37$, $p < 0.001$).

Compared to probe 1, rats were able to find the reward location when no visual cues were present (probe 3), but it took longer to complete the task. A planned comparison confirmed that latency was significantly longer in the proximal ($t_{(46)} = 4.40$, $p < 0.001$) and distal cue conditions ($t_{(46)} = 7.16$, $p < 0.001$), relative to probe 1, but performance was more severely impaired in the proximal cue condition. Although the rats were able to locate the food reward in probe 3, the higher latency scores suggest that place learning occurs through the combined use of egocentric strategies and visual cues.

In probe 2, in which the beacon was removed and a novel start point was used to assess allocentric strategies, performance was severely impaired compared to probe 1. Latency was near the maximum threshold of 30 seconds, indicating that rats often failed to locate the food reward. Compared to probe 1, it took rats longer to find the reward location in the proximal ($t_{(46)} = 8.73$, $p < 0.001$) and distal cue conditions ($t_{(46)} = 10.86$, $p < 0.001$), and again, performance was more severely impaired in the proximal task. The higher latency scores in this allocentric probe resulted from a large amount of time spent searching for the food reward in the wrong location, which suggests that egocentric strategies were used preferentially.

4. Discussion

4.1. Main Aims

The aim of this study was to examine the involvement of the anterior thalamic nuclei and laterodorsal thalamic nuclei in place learning using proximal and distal cues. The rationale behind this comparison was that previous work has shown that the anterior thalamic nuclei are involved in spatial memory processing, but there is little evidence for the contribution of the laterodorsal thalamic nuclei in similar tasks (Brett, 2008; Craw, et al., 2007; van Groen, et al., 2002b). Additionally, the proximity of visual cues has been shown to influence processing of spatial information (Brett, 2008; Cánovas, et al., 2011; Livingstone & Skelton, 2007; Save & Poucet, 2000), and there is evidence to suggest that place learning involves the use of both egocentric and allocentric navigation strategies (Klatzky, 1998; O'Keefe & Nadel, 1978).

This study used a novel spatial reference memory task which was designed to test the effect of cue proximity on the propensity to use egocentric or allocentric navigation strategies, and whether there was a change in strategies over time. This was achieved through the use of a fixed configuration of proximal or distal cues, a beacon indicating the location of the food reward, and a fixed start point relative to the visual cues and beacon. Egocentric strategies were encouraged through the use of the fixed trajectory, established by using the fixed relationship between the location of the beacon and the start point. Allocentric strategies were encouraged through the presence of a spatial cue configuration, which was created by either proximal or distal cues. Strategy use (egocentric or allocentric) was tested by repeating a set of three probes over a four week period. The expectations for this study are discussed in the following section (4.2).

4.2. Expectations

The testing regime comprised four components: acquisition and three probes. The following sections (4.2.1 to 4.2.4) outline the expectations for each component of the test in terms of A) performance over time, B) influence of cue proximity on performance, and C) performance of the AT and LD lesion groups relative to the sham lesion group. Performance was measured using deviation scores (a measure of navigation accuracy) and latency to locate the reward location. Performance in each probe emphasised the ability to use particular strategies, i.e. allocentric or egocentric navigation. Subsequently, the main findings are summarised (Section 4.3) and then the conclusions are discussed relative to the current literature (Section 4.4).

4.2.1. Acquisition

It was expected that during task acquisition, both latency and deviation scores to the food reward would decrease over time as the rats learnt the task. Performance at asymptote was expected to show longer latency in the proximal cue condition compared to the distal cue condition, but similar deviation scores across the two visual cue conditions. The first assumption is based on a previous study using the water maze (Brett, 2008), where latency was higher at asymptote when proximal cues were available to guide navigation compared to when distal cues were available. The second assumption is based on work by Save and Poucet (2000) who showed that rats with either hippocampal or parietal cortex lesions (presumed to be comparable to AT and LD lesions, respectively) could navigate toward a salient beacon in a water maze task. Both the sham and lesioned rats swam directly to the beacon indicating the use of a direct route. As such, it was assumed that rats would also use a direct route on the cheeseboard maze when the beacon indicated the location of the food reward, thus resulting in similar deviation scores for both lesion groups. The findings for acquisition are discussed in Section 4.3.1.

4.2.2. Probe 1: General Navigation

It was expected that the removal of the beacon, while using the same start point and same cue matrix to guide navigation, would result in a small initial increase in both deviation scores and latency, with a subsequent reduction over the four weeks for all rats as they learnt the task and adapted to this probe. Removal of the beacon was not expected to produce significant differences in performance between the two visual cue conditions, as the remaining cues were still available to support navigation. For the same reasons, disruption to performance was expected to be similar across the three lesion groups. The findings for probe 1 are discussed in Section 4.3.2.

4.2.3. Probe 2: Allocentric Navigation

Testing allocentric navigation through the use of a novel start point relative to the standard cue configuration was expected to result in an initial increase in both deviation scores and latency, with a subsequent reduction over the four weeks for all rats as they learnt the task. Both animal and human studies have shown poorer performance on navigation tasks when proximal cues were used than when using distal cues (Brett, 2008; Cánovas, et al., 2011). Thus, it was thought that when rats used proximal cues to guide navigation from a novel start point, performance would be poorer (higher deviation scores and longer latency) compared to navigation using distal cues on the cheeseboard maze. Previous work has shown that rats with AT lesions exhibit clear deficits on tasks that test allocentric navigation (Aggleton, et al., 1996; Wolff, et al., 2008), but rats with LD lesions exhibit mild or no impairments on similar allocentric tasks (Craw, et al., 2007; van Groen, et al., 2002b). Therefore, it was expected that performance of the AT lesion group would be severely impaired, while the LD lesion group would show mild to no impairment compared to the sham group. The findings for probe 2 are discussed in Section 4.3.3.

4.2.4. Probe 3: Egocentric Navigation

Removal of all visual cues was expected to result in an initial increase in both deviation scores and latency, with a subsequent reduction over the four weeks for all rats as they learnt the task. Because the visual cues were no longer available to guide navigation, it was expected that performance would reflect egocentric responding, irrespective of whether proximal or distal visual cues had previously guided navigation. Previous work has shown that rats with AT lesions do not exhibit impaired performance on egocentric tasks (Aggleton, et al., 1996; Sziklas & Petrides, 1999; Warburton, et al., 1997; Wolff, et al., 2008). Performance for the AT and sham groups were expected to remain unchanged. Although there is currently no literature on the performance of rats with LD lesions in egocentric tasks, it was expected that the LD lesion group would be impaired (higher deviation scores and longer latency) compared to the sham group. This assumption is based on the neural connections of the laterodorsal thalamic nuclei with the parietal cortex, a structure which is implicated in egocentric processing. The findings for probe 3 are discussed in Section 4.3.4.

4.3. Main Findings

4.3.1. Acquisition

There was an overall decrease in both deviation scores and latency across acquisition as the rats learnt to navigate to the food reward. At the start of acquisition training, deviation scores were higher in the proximal cue condition compared to the distal cue condition, and remained higher at asymptote. Latency was also marginally higher at the start of acquisition in the proximal cue condition, but then stabilised, resulting in similar latency at asymptote across the two visual cue conditions.

Sham and LD lesioned rats performed similarly, each with lower deviation scores than the AT lesion group. While performance was poorer for all three groups in the

proximal task, the AT group were more severely impaired. Latency to find the food reward was similar across the three lesion groups and the two visual cue conditions.

4.3.2. Probe 1: General Navigation

Relative to performance in standard acquisition trials, in which the beacon and all visual cues were intact, removal of the beacon from the cue matrix resulted in increased deviation scores and increased latency to locate the food reward across both proximal and distal cue conditions.

Overall, performance was poorer in the proximal cue condition, with higher deviation scores and longer latency, compared to the distal cue condition. There was, however, evidence of a small, but not significant, improvement in deviation scores across weeks 2 to 4 in both visual cue conditions (Figure 3.58), which suggests that either a change in navigation strategy was taking place or that the rats were adjusting to the removal of the beacon.

Compared to standard acquisition trials, the three lesion groups all exhibited higher deviation scores and longer latency to locate the food reward, with both measures higher in the proximal cue condition compared to the distal cue condition. There were no differences in deviation scores or latency in the LD lesion group relative to the sham lesion group in either visual cue condition. However, the AT lesion group showed poorer deviation scores compared to the sham lesion group in the distal cue condition, but exhibited similar deviation scores in the proximal condition, and similar latency to locate the food reward.

4.3.3. Probe 2: Allocentric Navigation

Relative to performance in standard acquisition trials, in which the beacon and all visual cues were intact, removal of the beacon and the use of a novel start point resulted in higher deviation scores and longer latency to locate the food reward across both proximal and distal cue conditions. There was no improvement in deviation scores or latency across the

four weeks of probes which indicates that there was no modification of navigation strategies over time.

Overall, latency was longer in the proximal cue condition compared to the distal cue condition. The deviation scores were marginally poorer in the proximal cue condition compared to the distal cue condition when the standard scoring system was used. When the modified scoring system was used, the differences between the visual cue conditions were exaggerated. The three lesion groups exhibited similar deviation scores across both visual cue conditions when the standard scoring system was employed (see Figure 3.58). However, when the modified scoring system was used, performance was clearly poorer for all groups in the proximal cue condition, with higher deviation scores and longer latency, compared to the distal cue condition.

4.3.4. Probe 3: Egocentric Navigation

Relative to performance in standard acquisition trials, in which the beacon and all visual cues were intact, removal of all visual cues resulted in higher deviation scores and latency to locate the food reward across both proximal and distal cue conditions.

Overall, performance was poorer in the proximal cue condition, with higher deviation scores and longer latency, compared to the distal cue condition. There was no improvement in deviation scores or latency across the four weeks of probes which indicates that there was no modification of navigation strategies over time.

Compared to standard acquisition trials, the three lesion groups all exhibited higher deviation scores and longer latency to locate the food reward with both measures higher in the proximal cue condition compared to the distal cue condition. There were no differences in deviation scores between the AT and LD lesion groups, relative to the sham lesion group, in either visual cue condition. However, compared to the sham lesion group, longer latency was observed in the LD lesion group while the AT lesion group showed intermediate performance in the proximal cue condition only.

4.4. Conclusions and Considerations

4.4.1. Acquisition

As expected, both deviation scores and latency decreased across standard acquisition trials on the cheeseboard task. Thus, navigating to a specific place identified by a beacon became more efficient with repeated training. Previous work found that performance was more accurate (lower latency) in a water maze task at asymptote when distal cues facilitated navigation compared to when proximal cues were available (Brett, 2008). In contrast, in this study, latency was found to be similar at asymptote in the proximal and distal cue conditions. This could be due to the difference in the task parameters, i.e. in this study navigation was guided by a beacon and hanging visual cues, whereas in the water maze task, navigation was guided solely by hanging proximal cues. Other factors that may have influenced these task differences include motor activity (swimming versus running), food intake (free-feeding versus food-restricted) and the reinforcing reward (aversive versus appetitive). To illustrate the differences in performance between two identical tests, with the only difference being one was water and the other dry, Kant, et al., (1988) directly compared latency using food as a reward in the dry version, and an escape platform as a reward in the water version, and found that food-restricted rats learnt to solve the water maze task more quickly than the dry-maze maze task, and they learnt both tasks faster than the free-feeding rats.

It is well known that lesioned rats are able to navigate towards a salient beacon (Jarrard, 1983; Save & Poucet, 2000; Whishaw, Cassel, & Jarrad, 1995). However, in this study, the proximity of the visual cues influenced performance, as demonstrated by the higher deviation scores in the proximal cue condition compared to the distal cue condition. This indicates that even when a salient beacon revealed the location of a specific place, external cues had an influence on navigation. Moreover, in support of current literature, the relative proximity of the visual cues was critical to guiding navigation, as poorer

performance was consistently observed when the cues were more proximal than distal relative to the subject (Cánovas, et al., 2011; Save & Poucet, 2000).

It was expected that during acquisition, the three lesion groups would perform similarly to each other in both visual cue conditions. However, the AT lesion group showed higher deviation scores, but similar latency, relative to the sham and LD lesion groups across both visual cue conditions. This indicates that rats with AT lesions are able to locate a specific place using the beacon, but their ability to navigate accurately was diminished.

4.4.2. Probes

Each probe was designed to assess either a general, egocentric or allocentric navigation strategy. Performance in each probe emphasised the ability to use particular strategies. As expected, there was a clear difference between performance across acquisition, and performance for each probe. All probe trials resulted in both higher deviation scores and longer latency to locate the reward location. These changes in performance were more pronounced in the proximal cue condition compared to the distal cue condition. The sham lesioned rats did not show the expected results, particularly in the allocentric probe, which made it difficult to draw any conclusions with regard to the effect of AT or LD lesions on place learning.

Probe 1 tested general navigation through removal of the beacon, and using the same start point and same cue matrix setup as acquisition. Sham rats tended to head in the general direction of the learned trajectory (i.e. left or right), indicative of egocentric navigation. Subsequently, instead of using the spatial configuration to find the reward location, where localised searching should be visible, individual cues appeared to be used as a guide, resulting in higher deviation scores, as the remaining visual cues did not indicate the specific location of the reward (see Figures 3.17 and 3.21). Longer latencies were also observed, as poorer search strategies were used to locate the reward. These

results suggest that the beacon, but not the spatial configuration, was used to guide navigation. Poorer performance was more pronounced when proximal cues were available than when distal cues were available for both the sham and LD lesion groups. As expected, the LD lesion group performed similarly to the sham group across both measures. In contrast, the AT lesion group showed less accurate navigation when distal cues, but not proximal cues, were available. Despite this mild lesion effect, it was surprising that the three groups showed such high deviation scores overall relative to standard acquisition trials. It is probable that the beacon was an essential part of the cue matrix and influenced heading direction. As shown by the trajectories in Figures 3.17 and 3.21, removal of the beacon likely produced a shift in focus to the cues that had previously been close to the beacon. These then became the salient cues that guided navigation instead of the spatial cue configuration.

Probe 2 tested allocentric navigation through removal of the beacon and use of a novel start point relative to the standard cue configuration. Similar to probe 1, sham rats tended to head in the general direction of the learned trajectory (i.e. left or right), indicative of egocentric, not allocentric navigation. Again, instead of using the spatial configuration to find the reward location, individual cues appeared to be used to guide navigation (see Figures 3.26 and 3.30). As the food reward was not, in this case, located in the same general direction, deviation scores were high and the latency to locate the food reward was near the maximum threshold. This was surprising, as previous work has shown that sham lesioned rats are able to locate a hidden platform in a water maze task using allocentric strategies (Eichenbaum, et al., 1990; Wolff, et al., 2008). As mentioned in Section 4.4.1, the discrepancies between the water maze task and this cheeseboard maze task may be due to differences in task conditions including motor activities, food intake and the reward type or the continued use of a salient beacon during acquisition. Furthermore, the prediction that the AT lesion group would exhibit poorer performance than the sham and LD lesion

groups was unable to be tested as the sham group failed to show the expected allocentric navigation strategy. These results are not consistent with literature work, and likely arose from the increased focus on the beacon to guide navigation, rather than the overall spatial configuration.

Probe 3 tested egocentric navigation through the removal of all visual cues. Similar to probes 1 and 2, sham rats tended to head in the general direction of the learned trajectory (i.e. left or right), indicative of egocentric navigation. However, this trajectory was maintained for only for a short distance before deviating from the reward location. This resulted in higher deviation scores (see Figures 3.50 and 3.54). Longer latencies were also observed, as poorer search strategies were used to locate the reward. This contrasts with previous results, which found sham rats were able to navigate to a hidden platform in a water maze task using egocentric strategies exclusively (Mogensen, Moustgaard, Khan, Wortwein, & Nielsen, 2005). Again, the discrepancies between the water maze task and this cheeseboard maze task may be due to differences in task conditions or the continued use of a salient beacon during acquisition. As seen in Figures 3.50 and 3.54, both lesion groups exhibited similar trajectory patterns as the sham lesion group, with a correct initial heading direction followed by deviation away from the reward location, indicative of egocentric navigation. It was expected that because the laterodorsal thalamic nuclei have neural connections with the parietal cortex, and this structure is involved in processing egocentric navigation, the LD lesion group would show poorer deviation scores and longer latency compared to the sham and AT lesion groups when navigation relied solely on self-generated egocentric responses. However, this was clearly not supported, despite the LD lesion group exhibiting marginally longer latency compared to sham lesion rats when proximal cues, but not distal cues, had previously been available to guide navigation. This suggests that the connections between the laterodorsal thalamic nuclei and the parietal cortex are not critical for processing egocentric navigation.

The clear discrepancy in performance between proximal and distal cues raises the issue of the effect cue proximity has on spatial navigation and the propensity to use egocentric or allocentric navigation strategies. These effects could be due to a combination of motion parallax and a preference for the use of cued versus place navigation.

Cues that are closer to the subject appear to be displaced faster than cues that are further away, which appear stationary, known as motion parallax (Gibson, Gibson, Smith, & Flock, 1959). For example, when driving in a car, nearby objects such as trees are displaced quickly, but distant mountains appear relatively static. The act of moving past nearby objects requires a subject to rapidly update their cognitive map as the position of the cues relative to each other, the reward location, and the subject, changes in location. In contrast, the position of distant cues relative to each other, the reward target, and a moving subject appear static, and therefore the cognitive map requires less frequent updating (O'Keefe & Nadel, 1978). Consequently, navigation using proximal cues generates a much higher cognitive load, which, in this study, may have resulted in poorer performance than navigation using distal cues.

Rats were required to navigate to a beacon which directly indicated the location of the food reward (cued navigation). In addition, a spatial cue configuration of either proximal or distal cues was available, but did not directly indicate the reward location (place navigation). It was assumed that the rats would run to the beacon and attend to the spatial cue configuration to build up a spatial representation of the environment. However, the results showed that the information from cued and place navigation was not integrated. Instead, cued navigation was used preferentially with rats running in the direction of the learned trajectory. In particular, in probe 2, which tested allocentric strategies, high deviation scores were observed which corresponded to the use of egocentric strategies. However, the visual cues did influence performance, indicating that they were attended to, to some degree. However, based on the trajectories seen in Figures 3.26 and 3.30, it is

more likely that individual cues, rather than the spatial configuration facilitated locating the reward place.

As discussed in Section 1.2 the neural structures that process cued and place navigation differ. Cued navigation is processed primarily by the parietal and occipital cortices whereas place navigation is processed primarily by the medial temporal lobe, which has clear connections with the diencephalon (extended hippocampal formation) (Aggleton, 2008; Aggleton & Brown, 1999). Therefore, if cued navigation was used preferentially over place navigation, the extended hippocampal formation would not have been involved in processing information used for cued navigation, and this could explain the high deviation scores and longer latency seen, in particular, in the allocentric navigation probe. Because cued navigation is processed by the parietal cortex, performance deficits should have emerged in the LD lesion group. However, this was not evident, suggesting that the connections between the parietal cortex and laterodorsal thalamic nuclei and not essential for processing egocentric responses.

Spatial memory studies in the water maze typically involve learning the location of a hidden escape platform using only distal room cues from four cardinal start points. Rats are tested on recall of the platform location using the same start points. One disadvantage of this procedure is that rats learn to use non-spatial strategies, gained from four viewpoints, to locate the platform (e.g. the distance of the escape platform from the pool edge). To counter this, a study by Eichenbaum, et al., (1990) used the same basic procedures described above, but trained rats to locate the hidden platform using a single constant start point and then tested them from novel start points. This encouraged the association of distal cues with a fixed trajectory and an escape reward. A single constant start point also reduced non-spatial strategies by restricting the number of viewpoints to one. Release from novel start points tested whether rats were able to create and use a spatial representation of the environment after navigating from a single trajectory. Control rats were able to use the

representation of the environment learned during training to locate the escape platform from a novel start point, but rats with fornix lesions exhibited severe deficits. During training, the platform initially sat above the water with the visibility of the escape platform gradually faded out over the course of the study (Eichenbaum, et al., 1990). In comparison, in this study, constant use of the beacon to encourage a fixed trajectory may have discouraged the formation of a spatial representation of the visual cues. This may then explain the preferential use of egocentric strategies when tested from novel start points in probe 2 in the current study.

Another possible limitation in this study is that rats were not restrained on the maze edge prior to running. In contrast, previous land-based maze studies have used a clear Perspex 'start box' which encourages the subjects to attend to the maze (Gilbert & Kesner, 2002; Gilbert, et al., 1998). The absence of a start box may have encouraged rats to run instinctively before attending to the environment, thus contributing to the prolific use of egocentric strategies exhibited in probe 2.

In summary, this study found that there were clear differences in performance between the use of proximal and distal cues to guide navigation, with consistently poorer performance when cues were closer to the subject. Lesion effects were generally not observed across the three probes, but this is likely due to the preferential use of cued navigation and the lack of integration between cued and place navigation. As such, it was likely that the parietal and occipital cortices were involved preferentially over the extended hippocampal system. Although it was thought that the connection between the laterodorsal thalamic nuclei and parietal cortex would impair egocentric navigation performance in the LD lesion group, the results suggest that this connection may not be critical for egocentric processing. However, because the LD lesions were relatively small in this study, lesion effects may not have been observed, so larger lesions may be needed. When general navigation was tested in probe 1, there was evidence of a slight improvement in navigation

strategy over the four weeks of testing, but little evidence of improvement when tested for allocentric and egocentric strategies in the subsequent probes. This may be because the rats were unable to associate the fixed trajectory and the spatial configuration with the location of the food reward.

5. Future Work

Given the results of this study, it would be valuable to repeat the task with a few modifications. For instance, instead of maintaining the beacon as the reward indicator throughout training, it may be beneficial to fade it out over one week, similar to the method of Eichenbaum, et al., (1990). This would encourage a strategy shift from cued navigation, thought to preferentially involve the parietal and occipital cortices, to place navigation where the spatial configuration would now be processed primarily by the extended hippocampal system. These would likely bring the results in line with the current spatial navigation literature with poor performance exhibited by rats with anterior thalamic lesions when tested for allocentric strategies but unimpaired when tested for egocentric strategies. It would also provide further evidence for the involvement of the laterodorsal thalamic nuclei in spatial memory processing. In addition to the maintained use of the beacon across training, a major limitation in this study was the poor lesion size in the laterodorsal thalamic region. The reasons behind this are unclear, but further lesion studies need to be carried out to generate consistent selective laterodorsal thalamic lesions of adequate size.

As discussed in Section 4.4.1, use of different tasks, i.e. land-based versus water mazes, can produce clear differences in performance. Thus, it would be useful to compare this dry maze task, with the modifications noted above, with a water maze using the same methodology. Use of the same animals across both task types would also provide more robust evidence for the effects of task procedures on spatial learning. Furthermore, to make sure that the rats are able to perform a simple egocentric/allocentric task, it would be beneficial to run the same rats in a non-matching to place alternation T-maze test, as described in Section 1.2.

Differences between spatial navigation tasks may also result from sex differences. For example, in human studies of spatial navigation, male subjects are typically faster and more accurate when navigating than female subjects (Cánovas, et al., 2011). Furthermore,

in an animal study using a water maze task, it was evident that sex differences can vary depending on prior experience with non-spatial aspects of a task (Perrot-Sinal, Kostenuik, Ossenkopp, & Kavaliers, 1996). However, the majority of the rat studies discussed in this thesis use a single sex, with a large proportion using males. The use of a single sex in spatial navigation tasks does not provide a comprehensive view of the effects of spatial memory and learning. Therefore, it would be valuable to determine sex differences across spatial navigation studies by including both male and female subjects in future animal work.

In summary, this thesis provides support for the effects of cue proximity on spatial navigation performance where navigation guided by proximal cues is more difficult than distal cues. It also provides evidence for the preferential use of cued navigation over place navigation when a salient beacon is maintained as part of a cue matrix. The absence of thalamic lesion effects when cued rather than place navigation is used also lends support to the influence of different neural networks when the information attended to is not spatial. Unfortunately, this study did not further our understanding of the contribution of the laterodorsal thalamic nuclei in the processing of spatial information, but it has highlighted some caveats that need to be considered in future studies.

6. References

Aggleton, J. P. (2008). Understanding anterograde amnesia: disconnections and hidden lesions. *Quarterly Journal of Experimental Psychology, 61*(10), 1441-1471.

Aggleton, J. P., Amin, E., Jenkins, T. A., Pearce, J. M., & Robinson, J. (2011). Lesions in the anterior thalamic nuclei of rats do not disrupt acquisition of stimulus sequence learning. *The Quarterly Journal of Experimental Psychology, 64*(1), 65-73.

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal–anterior thalamic axis. *Behavioral And Brain Sciences, 22*, 425-489.

Aggleton, J. P., Hunt, P. R., Nagle, S., & Neave, N. (1996). The effects of selective lesions within the anterior thalamic nuclei on spatial memory in the rat. *Behavioural Brain Research, 81*(1-2), 189-198.

Aggleton, J. P., Neave, N., Nagle, S., & Hunt, P. R. (1995). A comparison of the effects of anterior thalamic, mamillary body and fornix lesions on reinforced spatial alternation. *Behavioural Brain Research, 68*(1), 91-101.

Aggleton, J. P., O'Mara, S. M., Vann, S. D., Wright, N. F., Tsanov, M., & Erichsen, J. T. (2010). Hippocampal-anterior thalamic pathways for memory: Uncovering a network of direct and indirect actions. *European Journal of Neuroscience, 31*(12), 2292-2307.

Aggleton, J. P., & Pearce, J. M. (2001). Neural systems underlying episodic memory: insights from animal research. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 356*(1413), 1467-1482.

Austin, J. H. (2003). Your Self, Your Brain, and Zen. Retrieved 14/04/2008, from http://www.dana.org/news/cerebrum/detail.aspx?id=2896

Baldi, E., Lorenzini, C. A., & Corrado, B. (2003). Task solving by procedural strategies in the Morris water maze. *Physiology & Behavior, 78*(4-5), 785-793.

Benhamou, S., & Poucet, B. (1998). Landmark use by navigating rats (Rattus norvegicus): Contrasting geometric and featural Information. *Journal of Comparative Psychology, 112*(3), 317-322.

Brett, F. M. (2008). *Thalamic lesion effects on proximal and distal spatial information processing: Anterior thalamic nuclei versus laterodorsal thalamic lesions.* Unpublished Research Report, University of Canterbury, Christchurch.

Burgess, N. (2008). Spatial cognition and the brain. *Annals of the New York Academy of Sciences, 1124*(1), 77-97.

Byatt, G., & Dalrymple-Alford, J. C. (1996). Both anteromedial and anteroventral thalamic lesions impair radial-maze learning in rats. *Behavioral Neuroscience, 110*(6), 1335-1348.

Cánovas, R., García, R. F., & Cimadevilla, J. M. (2011). Effect of reference frames and number of cues available on the spatial orientation of males and females in a virtual memory task. *Behavioural Brain Research, 216*(1), 116-121.

Chandler, H. C., King, V., Corwin, J. V., & Reep, R. L. (1992). Thalamocortical conncections of rat posterior parietal cortex. *Neuroscience Letters, 143*(1-2), 237-242.

Clayton, N. S., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature, 395*, 272-274.

Clearwater, J. M., & Bilkey, D. K. (2011). Place, space, and taste: Combining context and spatial information in a hippocampal navigation system. *Hippocampus, 21*, DOI: 10.1002/hipo.20911.

Cohen, M. D., & Bacdayan, P. (1994). Organizational routines are stored as procedural memory: Evidence from a laboratory study. *Organization Science, 5*(4), 554-568.

Corkin, S. (2002). What's new with the amnesic patient H.M.? *Nature Reviews. Neuroscience, 3*(2), 153-160.

Corkin, S., Amaral, R., González, G., Johnson, K. A., & Hyman, B. T. (1997). H. M.'s medial temporal lobe lesion: Findings from magnetic resonance imaging. *Journal of Neuroscience, 17*(10), 3964-3979.

Correll, R. E., & Scoville, W. B. (1965). Effects of medial temporal lesions on visual discrimination performance. *Journal of Comparative and Physiological Psychology, 60*(2), 175-181.

Craw, P. A., Rapley, S. A., Wolff, M., Kesner, R. P., & Dalrymple-Alford, J. C. (2007). *Anterior but not laterodorsal thalamic nuclei lesions impair continuous recognition in a radial arm maze and spatial reference memory in the Morris water maze*. Paper presented at the AWCBR Conference.

de Bruin, J. P. C., Moita, M. P., de Brabander, J. M., & Joosten, R. N. J. M. A. (2001). Place and response learning of rats in a Morris water maze: Differential effects of fimbria fornix and medial prefrontal cortex lesions. *Neurobiology of Learning and Memory, 75*, 164-178.

Dere, E., Kart-Teke, E., Huston, J. P., & De Souza Silva, M. A. (2006). The case for episodic memory in animals. *Neuroscience & Biobehavioral Reviews, 30*(8), 1206-1224.

Dumont, J. R., Petrides, M., & Sziklas, V. (2010). Fornix and retrosplenial contribution to a hippocampo-thalamic circuit underlying conditional learning. *Behavioural Brain Research, 209*(1), 13-20.

Eacott, M. J., & Easton, A. (2007). On familiarity and recall of events by rats. *Hippocampus, 17*, 890-897.

Eacott, M. J., Easton, A., & Zinkivskay, A. (2005). Recollection in an episodic-like memory task in the rat. *Learning & Memory, 12*, 221-223.

Eichenbaum, H. (2002). *The cognitive neuroscience of memory : An introduction* Oxford; New York: Oxford University Press.

Eichenbaum, H., Stewart, C., & Morris, R. G. M. (1990). Hippocampal representation in place learning. *Journal of Neuroscience, 10*(11), 3531-3542.

Faraji, J., Lehmann, H., Metz, G. A., & Sutherland, R. J. (2008). Rats with hippocampal lesion show impaired learning and memory in the ziggurat task: A new task to evaluate spatial behavior. *Behavioural Brain Research, 189*, 17-31.

Fleischman, D., & Gabrieli, J. (1999). Long-term memory in Alzheimer's disease. *Current Opinion In Neurobiology, 9*, 240-244.

Fouquet, C., Tobin, C., & Rondi-Reig, L. (2010). A new approach for modeling episodic memory from rodents to humans: The temporal order memory. *Behavioural Brain Research, 215*(2), 172-179.

Gibb, S. J., Wolff, M., & Dalrymple-Alford, J. C. (2006). Odour-place paired-associate learning and limbic thalamus: comparison of anterior, lateral and medial thalamic lesions. *Behavioural Brain Research, 172*, 155-168.

Gibson, E. J., Gibson, J. J., Smith, O. W., & Flock, H. (1959). Motion parallax as a determinant of perceived depth. *Journal of Experimental Psychology, 58*(1), 40-51.

Gilbert, P. E., & Kesner, R. P. (2002). Role of the rodent hippocampus in paired-associate learning involving associations between a stimulus and a spatial location. *Behavioral Neuroscience, 116*(1), 63-71.

Gilbert, P. E., Kesner, R. P., & DeCoteau, W. E. (1998). Memory for spatial location: Role of the hippocampus in mediating spatial pattern separation. *Journal of Neuroscience, 18*(2), 804-810.

Gold, J. J., & Squire, L. R. (2006). The anatomy of amnesia: Neurohistological analysis of three new cases. *Learning & Memory, 13*(6), 699-710.

Hamilton, D. A., Rosenfelt, C. S., & Whishaw, I. Q. (2004). Sequential control of navigation by locale and taxon cues in the Morris water task. *Behavioural Brain Research, 154*(2), 385-397.

Henry, J., Petrides, M., St-Laurent, M., & Sziklas, V. (2004). Spatial conditional associative learning: effects of thalamo-hippocampal disconnection in rats. *Learning & Memory, 15*(15), 2427-2431.

Horikawa, K., Kinjo, N., Stanley, L. C., & Powell, E. W. (1988). Topographic organization and collateralization of the projections of the anterior and laterodorsal thalamic nuclei to cingulate areas 24 and 29 in the rat. *Neuroscience Research, 6*(1), 31-44.

Jarrard, L. E. (1983). Selective hippocampal lesions and behavior: Effects of kainic acid lesions on performance of place and cue tasks. *Behavioral Neuroscience, 97*(6), 873-889.

Kant, G. J., Yen, M. H., D'Angelo, P. C., Brown, A. J., & Eggleston, T. (1988). Maze performance: A direct comparison of food vs. water mazes. *Pharmacology Biochemistry and Behavior, 31*(2), 487-491.

Klatzky, R. L. (1998). *Allocentric and egocentric spatial representations: Definitions, distinctions, and interconnections*. Paper presented at the Spatial cognition, an interdisciplinary approach to representing and processing spatial knowledge.

Kolb, B., & Walkey, J. (1987). Behavioural and anatomical studies of the posterior parietal cortex in the rat. *Behavioural Brain Research, 23*(2), 127-145.

Kopelman, M. D., Thomson, A. D., Guerrini, I., & Marshall, E. J. (2009). The Korsakoff syndrome: Clinical aspects, psychology and treatment. *Alcohol and Alcoholism, 44*(2), 148-154.

Lezak, M. D., Howieson, D. B., & Loring, D. W. (2004). *Neurological assessment* (Fourth ed.): Oxford: University Press.

Livingstone, S. A., & Skelton, R. W. (2007). Virtual environment navigation tasks and the assessment of cognitive deficits in individuals with brain injury. *Behavioural Brain Research, 185*(1), 21-31.

Lopez, J., Wolff, M., Lecourtier, L., Cosquer, B., Bontempi, B., Dalrymple-Alford, J., et al. (2009). The intralaminar thalamic nuclei contribute to remote spatial memory. *Journal of Neuroscience, 29*(10), 3302-3306.

Mair, R. G., Burk, J. A., & Porter, M. C. (1998). Lesions of the frontal cortex, hippocampus, and intralaminar thalamic nuclei have distinct effects on remembering in rats. *Behavioral Neuroscience, 112*(4), 772-792.

Mair, R. G., Burk, J. A., & Porter, M. C. (2003). Impairment of radial maze delayed nonmatching after lesions of anterior thalamus and parahippocampal cortex. *Behavioral Neuroscience, 117*(3), 596-605.

Mitchell, A. S., & Dalrymple-Alford, J. C. (2005). Dissociable memory effects after medial thalamus lesions in the rat. *European Journal Of Neuroscience, 22*, 973-985.

Mitchell, A. S., & Dalrymple-Alford, J. C. (2006). Lateral and anterior thalamic lesions impair independent memory systems. *Learning & Memory, 13*(3), 388-396.

Mizumori, S. J., Miya, D. Y., & Ward, K. E. (1994). Reversible inactivation of the lateral dorsal thalamus disrupts hippocampal place representation and impairs spatial learning. *Brain Research, 644*(1), 168-174.

Mizumori, S. J., & Williams, J. D. (1993). Directionally selective mnemonic properties of neurons in the lateral dorsal nucleus of the thalamus of rats. *Journal of Neuroscience, 13*(9), 4015-4028.

Mogensen, J., Moustgaard, A., Khan, U., Wortwein, G., & Nielsen, K. S. (2005). Egocentric spatial orientation in a water maze by rats subjectd to transection of the fimbria-fornix and/or ablation of the prefrontal cortex. *Brain Research Bulletin, 65*, 41-58.

Moran, J. P., & Dalrymple-Alford, J. C. (2003). Perirhinal cortex and anterior thalamic lesions: comparative effects on learning and memory. *Behavioral Neuroscience, 117*(6), 1326-1341.

O'Keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map*. Oxford, UK: Clarendon Press.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). San Diego: Academic Press.

Perrot-Sinal, T. S., Kostenuik, M. A., Ossenkopp, K. P., & Kavaliers, M. (1996). Sex differences in performance in the Morris water maze and the effects of initial nonstationary hidden platform training. *Behavioral Neuroscience, 110*(6), 1309-1320.

Reece, M., & Harris, K. D. (1996). Memory for places: A navigational model in support of Marr's theory of hippocampal function. *Hippocampus, 6*, 735-748.

Reep, R. L., Chandler, H. C., King, V., & Corwin, J. V. (1994). Rat posterior parietal cortex - Topography of corticocortical and thalamic connections. *Experimental Brain Research, 100*(1), 67-84.

Renaudineau, S., Poucet, B., & Save, E. (2007). Flexible use of proximal objects and distal cues by hippocampal place cells. *Hippocampus, 17*, 381-395.

Rondi-Reig, L., Petit, G. H., Tobin, C., Tonegawa, S., Mariani, J., & Berthoz, A. (2006). Impaired sequential egocentric and allocentric memories in forebrain-specific–NMDA receptor knock-out mice during a new task dissociating strategies of navigation. *Journal of Neuroscience, 26*(15), 4071-4081.

Rossier, J., Grobety, M. C., & Schenk, F. (2000). Spatial learning by rats across visually disconnected environments. *Animal Learning & Behavior, 28*(1), 16-27.

Ryszka, A., & Heger, M. (1979). Afferent connections of the laterodorsal thalamic nucleus in the rat. *Neuroscience Letters, 15*(1), 61-64.

Save, E., & Moghaddam, M. (1996). Effects of lesions of the associative parietal cortex on the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat. *Behavioral Neuroscience, 110*(1), 74-85.

Save, E., & Poucet, B. (2000). Involvement of the hippocampus and associative parietal cortex in the use of proximal and distal landmarks for navigation. *Behavioural Brain Research, 109*, 195-206.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery & Psychiatry 20*, 11-21.

Scoville, W. B., & Milner, B. (2000). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neuropsychiatry & Clinical Neurosciences, 12*(1), 103a-113.

Shapiro, M. L., Tanila, H., & Eichenbaum, H. (1997). Cues that hippocampal place cells encode: dynamic and hierarchical representation of local and distal stimuli. *Hippocampus, 7*(6), 624-642.

Shibata, H. (2000). Organization of retrosplenial cortical projections to the laterodorsal thalamic nucleus in the rat. *Neuroscience Research, 38*, 303-311.

Shibata, H., & Naito, J. (2005). Organization of anterior cingulate and frontal cortical projections to the anterior and laterodorsal thalamic nuclei in the rat. *Brain Research, 1059*, 93-103.

Shinkai, M., Yokofujita, J., Oda, S., Murakami, K., Igarashi, H., & Kuroda, M. (2005). Dual axonal terminations from the retrosplenial and visual association cortices in the laterodorsal thalamic nucleus of the rat. *Anatomy and Embryology, 210*(4), 317.

Sikes, R. W., & Vogt, B. A. (1987). Afferent connections of anterior thalamus in rats: Sources and association with muscarinic acetylcholine receptors. *Journal of Comparative Neurology, 256*(4), 538-551.

Squire, L. R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychological Review, 99*(2), 195-231.

Stackman, R. W., & Taube, J. S. (1997). Firing properties of head direction cells in the rat anterior thalamic nucleus: Dependence on vestibular input. *Journal of Neuroscience, 17*(11), 4349-4358.

Sziklas, V., & Petrides, M. (1999). The effects of lesions to the anterior thalamic nuclei on object–place associations in rats. *European Journal of Neuroscience, 11*(2), 559-566.

Sziklas, V., & Petrides, M. (2007). Contribution of the anterior thalamic nuclei to conditional learning in rats. *Hippocampus, 17*, 456-461.

Taube, J. S. (1995). Head direction cells recorded in the anterior thalamic nuclei of freely moving rats. *Journal of Neuroscience, 15*, 70-86.

Taube, J. S. (1998). Head direction cells and the neurophysiological basis for a sense of direction. *Progress in Neurobiology, 55*, 225-256.

Touretzky, D. S., & Redish, A. D. (1996). Theory of rodent navigation based on interacting representations of space. *Hippocampus, 6*, 247-270.

Tulving, E. (2001). Episodic memory and common sense: how far apart? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 356*(1413), 1505-1515.

van Groen, T., Kadish, I., & Wyss, M. J. (1999). Efferent connections of the anteromedial nucleus of the thalamus of the rat. *Brain Research Reviews, 30*(1), 1-26.

van Groen, T., Kadish, I., & Wyss, M. J. (2002a). Role of the anterodorsal and anteroventral nuclei of the thalamus in spatial memory in the rat. *Behavioral And Brain Sciences, 132*, 19-28.

van Groen, T., Kadish, I., & Wyss, M. J. (2002b). The role of the laterodorsal nucleus of the thalamus in spatial learning and memory in the rat. *Behavioral And Brain Sciences, 136*, 239-337.

van Groen, T., & Wyss, M. J. (1992). Projections from the laterodorsal nucleus of the thalamus to the limbic and visual cortices in the rat. *Journal of Comparative Neurology, 324*(3), 427-448.

Vann, S. D., Aggleton, J. P., & Maguire, E. A. (2009). What does the retrosplenial cortex do? *Nature Reviews. Neuroscience, 10*(11), 792-802.

Vann, S. D., Honey, R. C., & Aggleton, J. P. (2003). Lesions of the mammillothalamic tract impair the acquisition of spatial but not nonspatial contextual conditional discriminations. *European Journal of Neuroscience, 18*(8), 2413-2416.

Vann, S. D., Saunders, R. C., & Aggleton, J. P. (2007). Distinct, parallel pathways link the medial mammillary bodies to the anterior thalamus in macaque monkeys. *European Journal of Neuroscience, 26*(6), 1575-1586.

Warburton, E. C., & Aggleton, J. P. (1999). Differential deficits in the Morris water maze following cytotoic lesions of the anterior thalamus and fornix transection. *Behavioural Brain Research, 98*(1), 27-38.

Warburton, E. C., Aggleton, J. P., & Muir, J. L. (1998). Comparing the effects of selective cingulate cortex lesions and cingulum bundle lesions on water maze performance by rats. *European Journal of Neuroscience, 10*(2), 622-634.

Warburton, E. C., Baird, A. L., & Aggleton, J. P. (1997). Assessing the magnitude of the allocentric spatial deficit associated with complete loss of the anterior thalamic nuclei in rats. *Behavioural Brain Research, 87*, 223-232.

Warburton, E. C., Baird, A. L., Morgan, A., Muir, J. L., & Aggleton, J. P. (2000). Disconnecting hippocampal projections to the anterior thalamus produces deficits on tests of spatial memory in rats. *European Journal of Neuroscience, 12*(5), 1714-1726.

Warburton, E. C., Baird, A. L., Morgan, A., Muir, J. L., & Aggleton, J. P. (2001). The conjoint importance of the hippocampus and anterior thalamic nuclei for allocentric spatial learning: Evidence from a disconnection study in the rat. *Journal of Neuroscience, 21*(18), 7323-7330.

Warburton, E. C., Morgan, A., Baird, A. L., Muir, J. L., & Aggleton, J. P. (1999). Does pretraining spare the spatial deficit associated with anterior thalamic damage in rats? *Behavioral Neuroscience, 113*(5), 956-967.

Whishaw, I. Q., Cassel, J. C., & Jarrad, L. E. (1995). Rats with fimbria-fornix lesions display a place response in a swimming pool: A dissociation between getting there and knowing where. *Journal of Neuroscience, 15*(8), 5779-5788.

Whishaw, I. Q., Hines, D. J., & Wallace, D. G. (2001). Dead reckoning (path integration) requires the hippocampal formation: evidence from spontaneous exploration and spatial learning tasks in light (allothetic) and dark (idiothetic) tests. *Behavioural Brain Research, 127*(1-2), 49-69.

Whishaw, I. Q., & Tomie, J. (1997). Perseveration on place reversal in spatial swimming pool tasks: Further evidence for place learning in hippocampal rats. *Hippocampus, 7*, 361-370.

Wilton, L. A. K., Baird, A. L., Muir, J. L., Honey, R. C., & Aggleton, J. P. (2001). Loss of thalamic nuclei for "head direction" impairs performance on spatial memory tasks in rats. *Behavioral Neuroscience, 115*(4), 861-869.

Wolff, M., Gibb, S. J., Cassel, J.-C., & Dalrymple-Alford, J. C. (2008). Anterior but not intralaminar thalamic nuclei support allocentric spatial memory. *Neurobiology of Learning and Memory, 90*(1), 71-80.

Wolff, M., Gibb, S. J., & Dalrymple-Alford, J. C. (2006). Beyond spatial memory: the anterior thalamus and memory for the temporal order of a sequence of odour cues. *Journal of Neuroscience, 26*, 2907-2913.

7. Appendices

Appendix A

7.1. Cresyl Violet Staining Protocol

A strict protocol was followed to ensure consistent colour stain across all brains.

Table 7.1. Cresyl Violet cell-body staining protocol.

	Step	Solution	Time/Dips
Delipidization	1	70% Ethanol	10 dips
	2	95% Ethanol	10 dips
	3	100% Ethanol	10 dips
	4	100% Ethanol	5 minutes
	5	95% Ethanol	10 dips
	6	70% Ethanol	5 minutes
Hydration	7	Distilled H_2O	1 minute
Stain	8	0.5% Cresyl Violet Solution	12 minutes
Rinsing	9	Distilled H_2O	2 minutes
	10	Distilled H_2O	2 minutes
Dehydration and Differentiation	11	70% Ethanol	2 minutes
	12	95% Ethanol	2 minutes
	13	95% Acid Alcohol*	40 seconds
	14	100% Ethanol	4 minutes
	15	100% Ethanol	4 minutes
Cleaning	16	Xylene	5 minutes
	17	Xylene	5 minutes
Mounting Coverslips	18	Depex	

*Acid alcohol – 400 mL 95% ethanol + 1 mL Glacial acetic acid

Appendix B

7.2. Anterior Thalamic Lesion diagrams

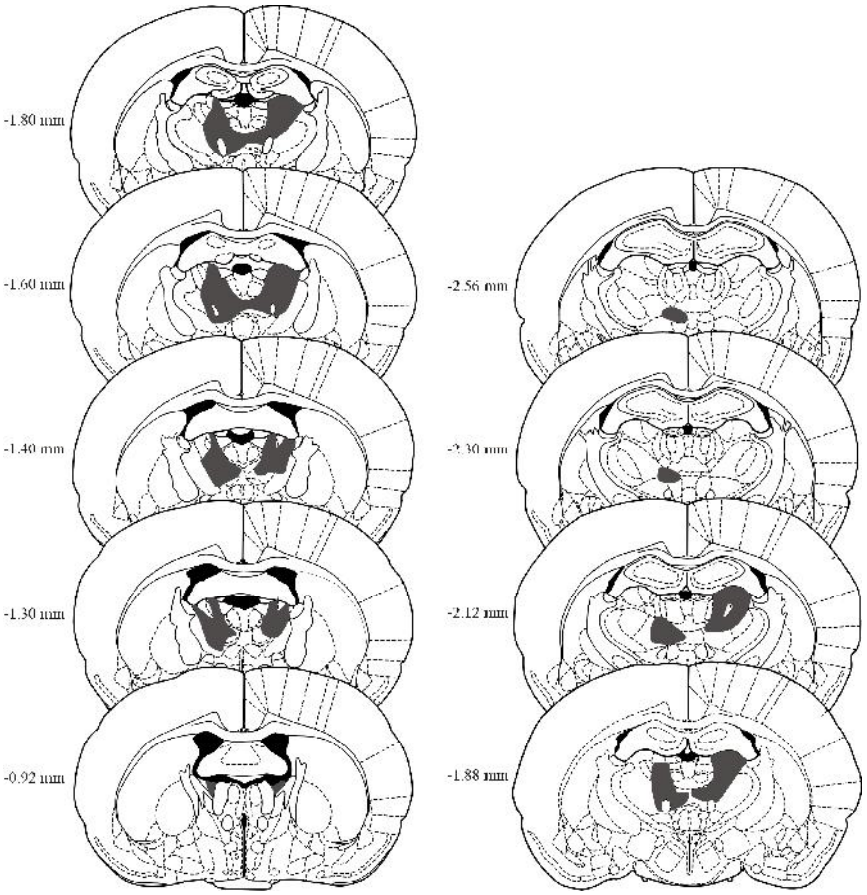

Figure 7.1. Rat #04 D-R. AT volume damage, 76%; LD volume damage, 6%.

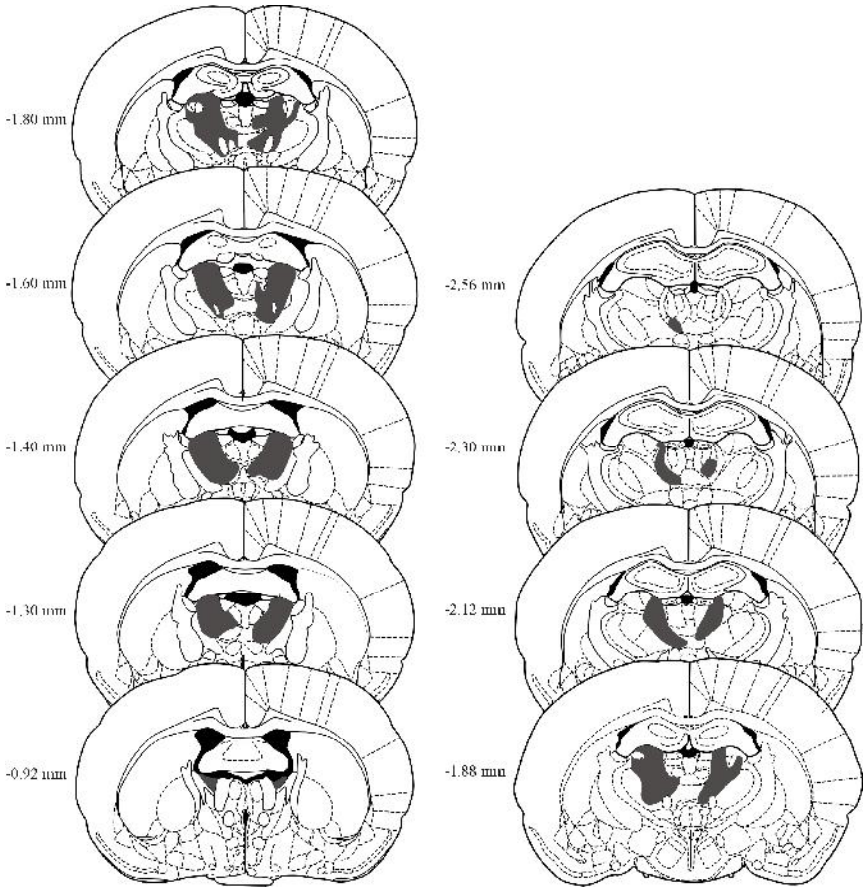

Figure 7.2. Rat #07 H-B. AT volume damage, 86%; LD volume damage, 3%.

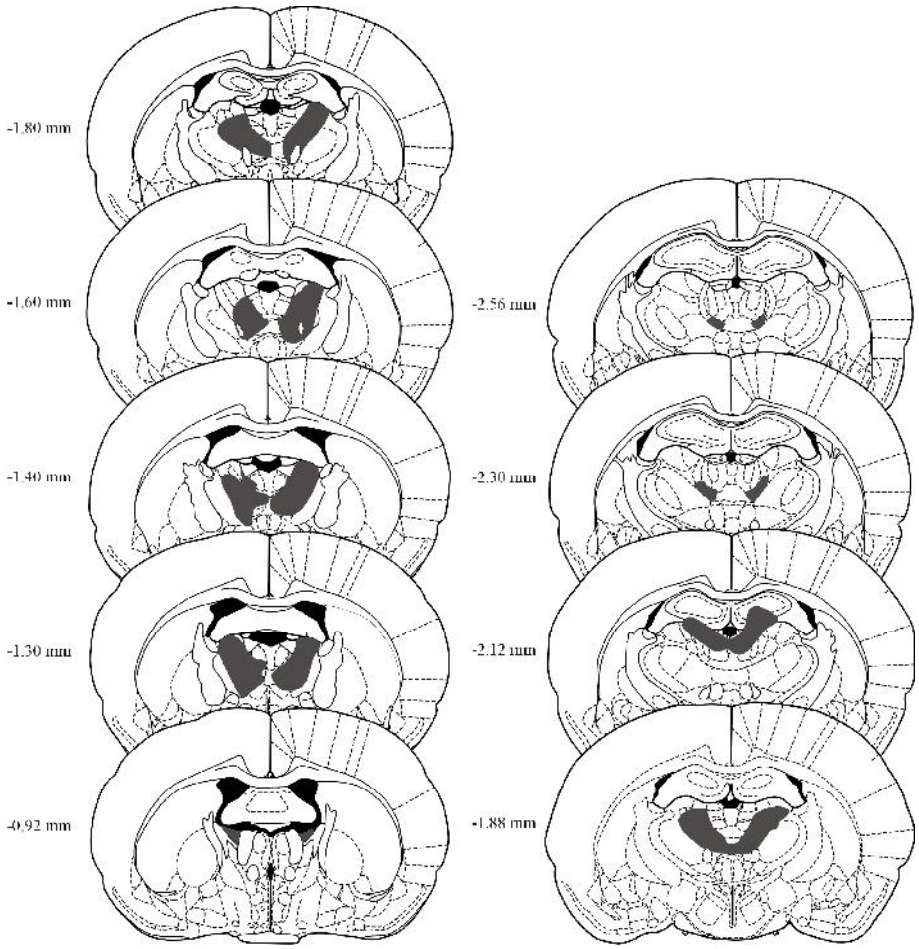

Figure 7.3. Rat #09 J-R. AT volume damage, 80%; LD volume damage, 1%.

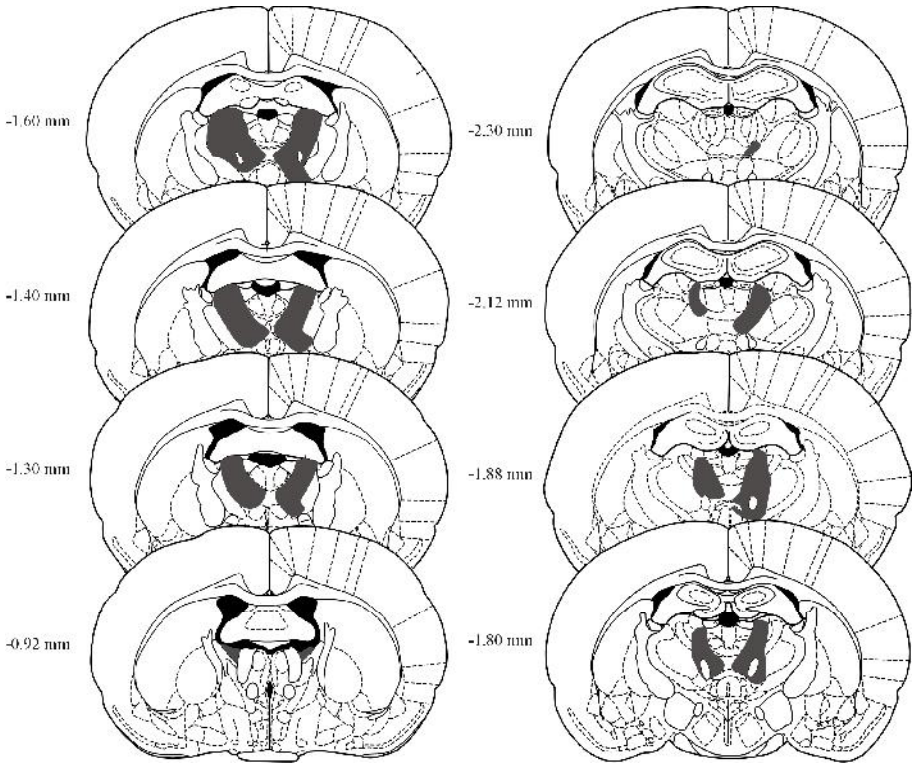

Figure 7.4. Rat #13 O-G. AT volume damage, 84%; LD volume damage, 0%.

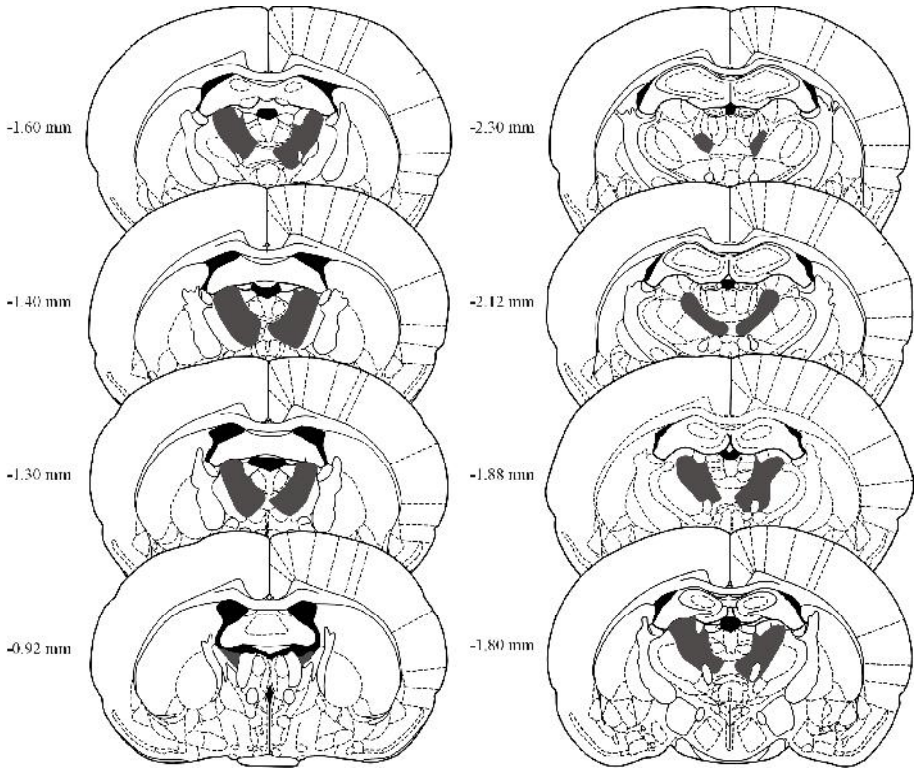

Figure 7.5. Rat #18 D-G. AT volume damage, 89%; LD volume damage, 2%.

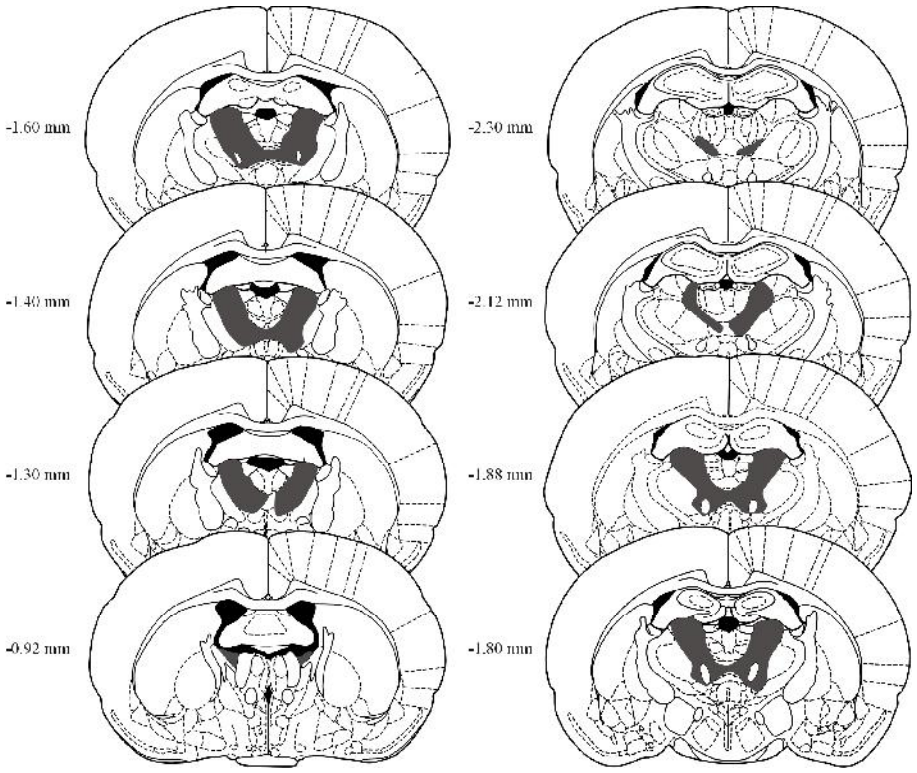

Figure 7.6. Rat #25 M-N. AT volume damage, 97%; LD volume damage, 5%.

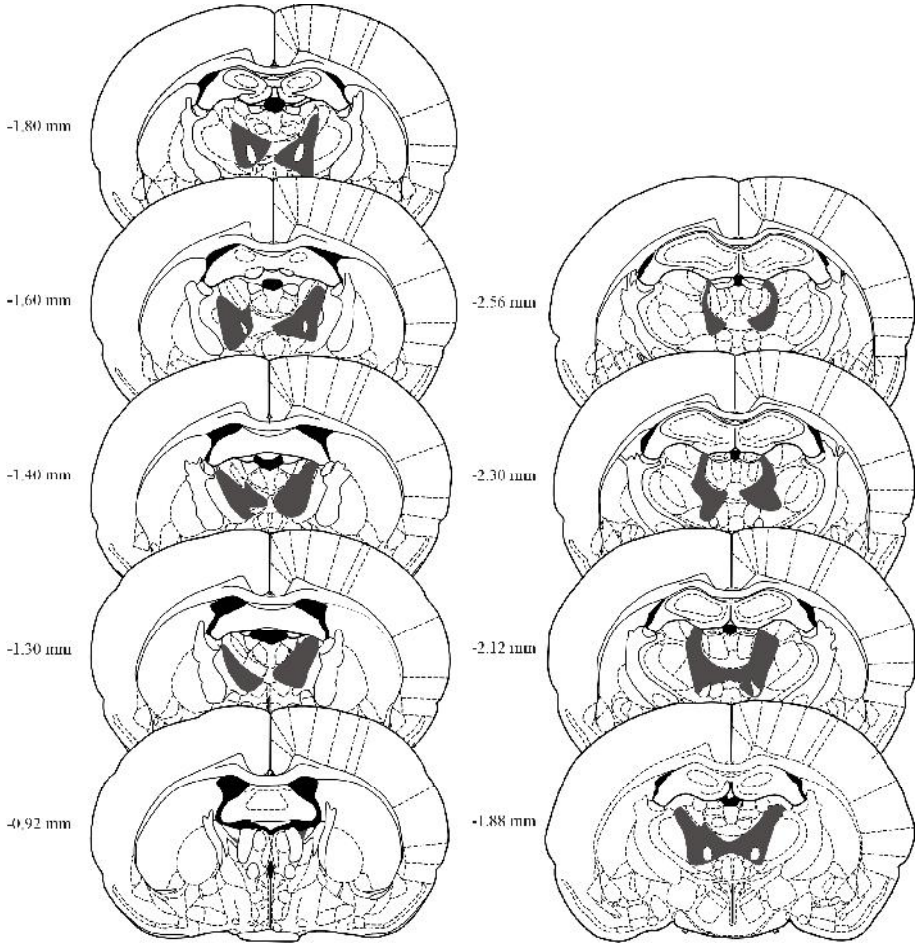

Figure 7.7. Rat #27 P-R. AT volume damage, 61%; LD volume damage, 1%.

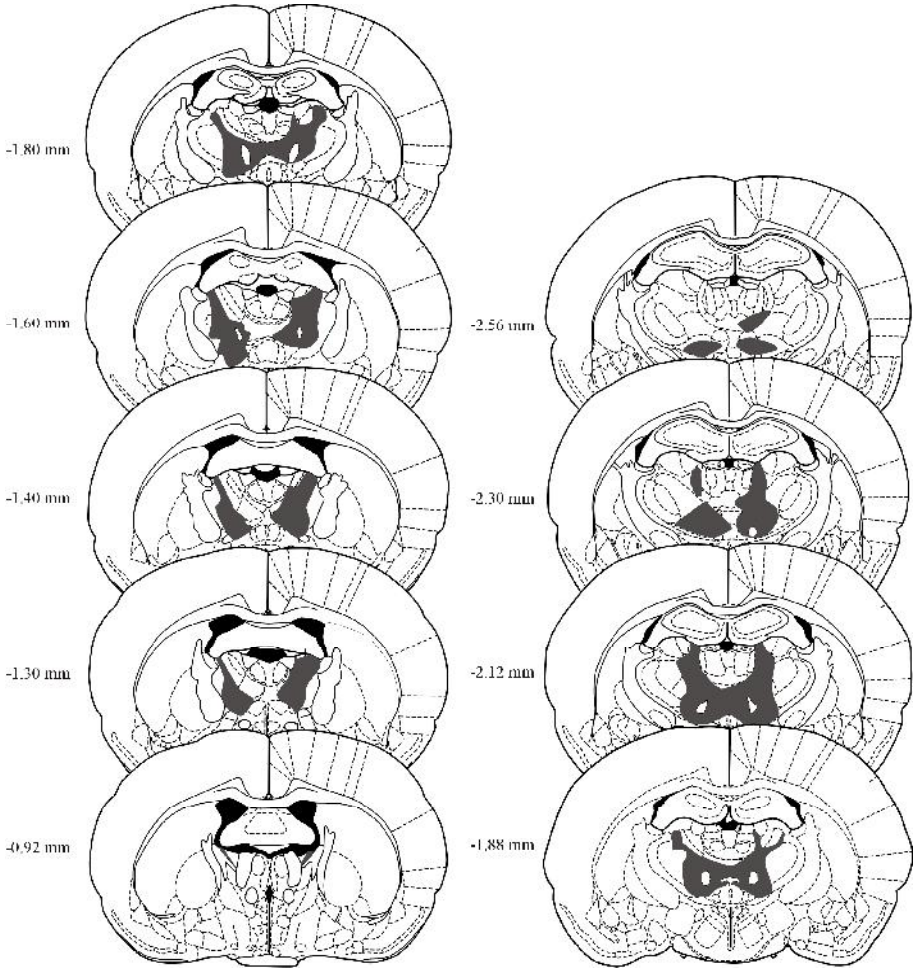

Figure 7.8. Rat #30 F-R. AT volume damage, 54%; LD volume damage, 1%.

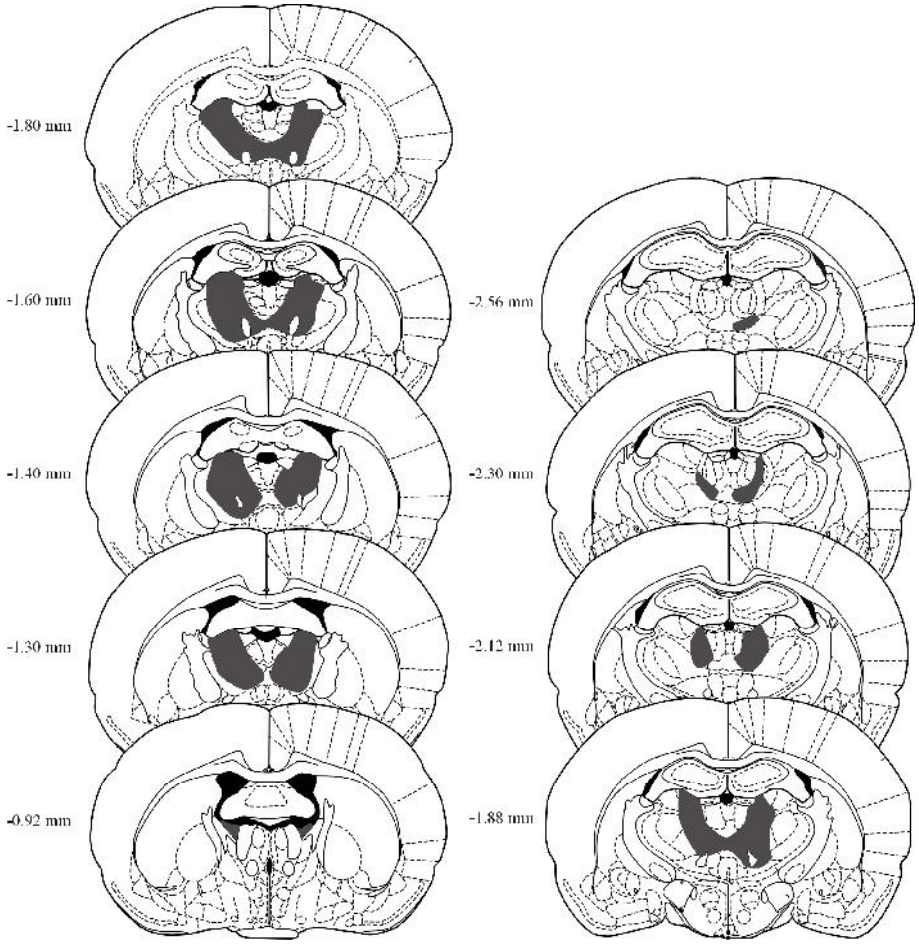

Figure 7.9. Rat #32 J-G. AT volume damage, 96%; LD volume damage, 5%.

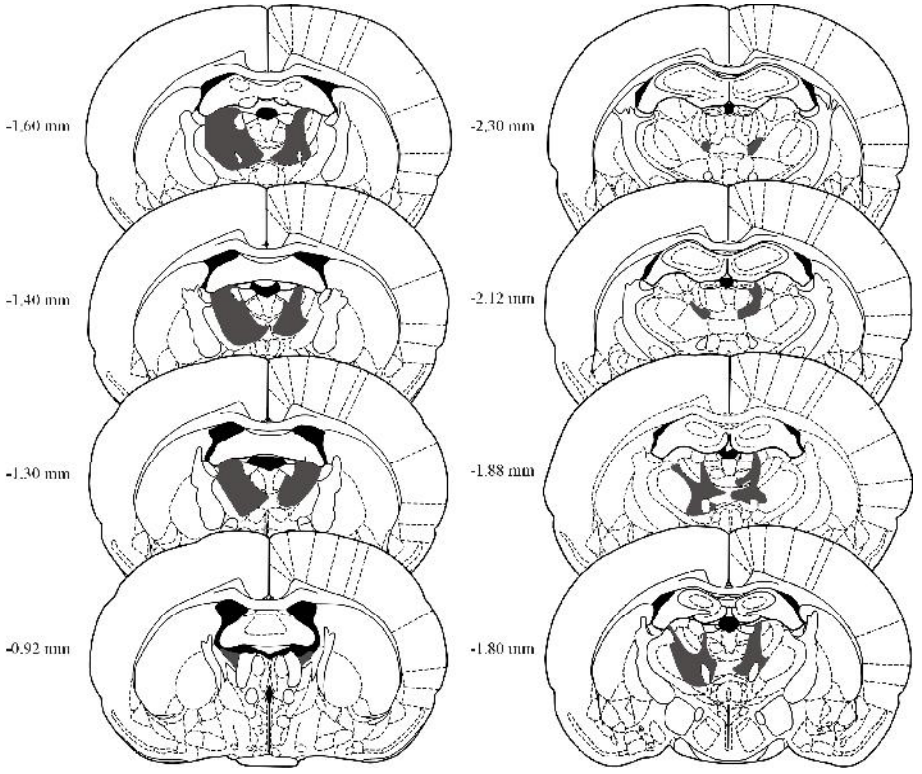

Figure 7.10. Rat #35 L-G. AT volume damage, 67%; LD volume damage, 0%.

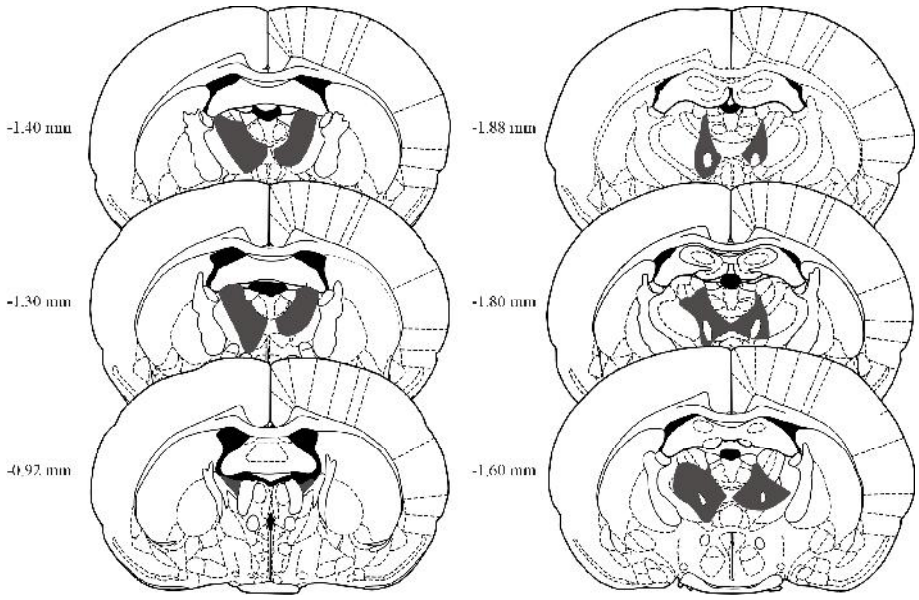

Figure 7.11. Rat #47 Ly-N. AT volume damage, 67%; LD volume damage, 0%.

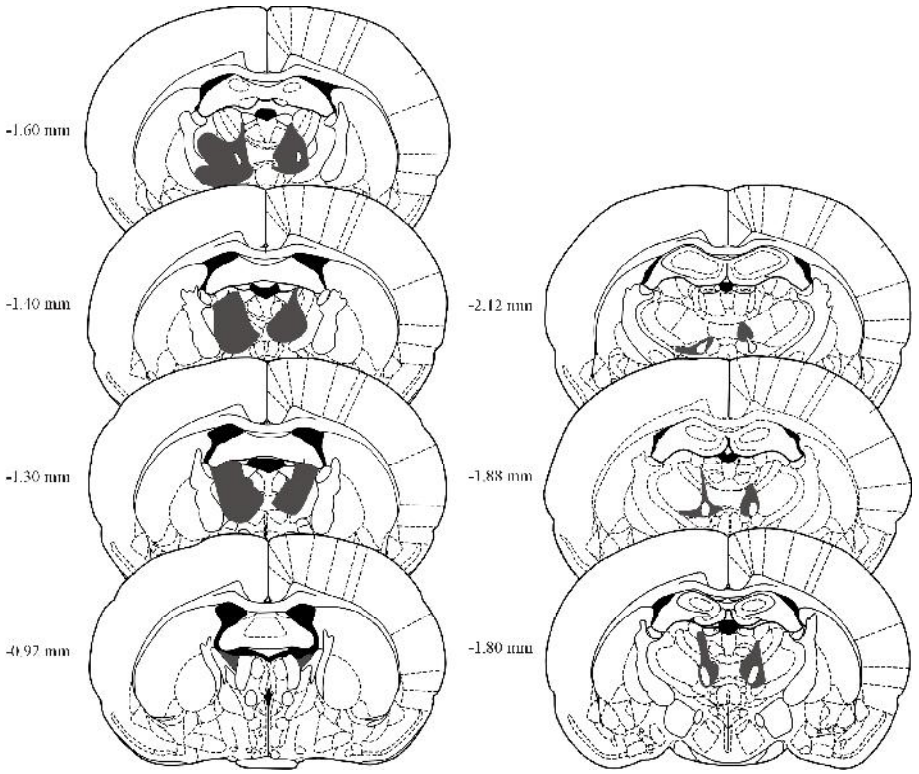

Figure 7.12. Rat #56 Te-N. AT volume damage, 56%; LD volume damage, 0%.

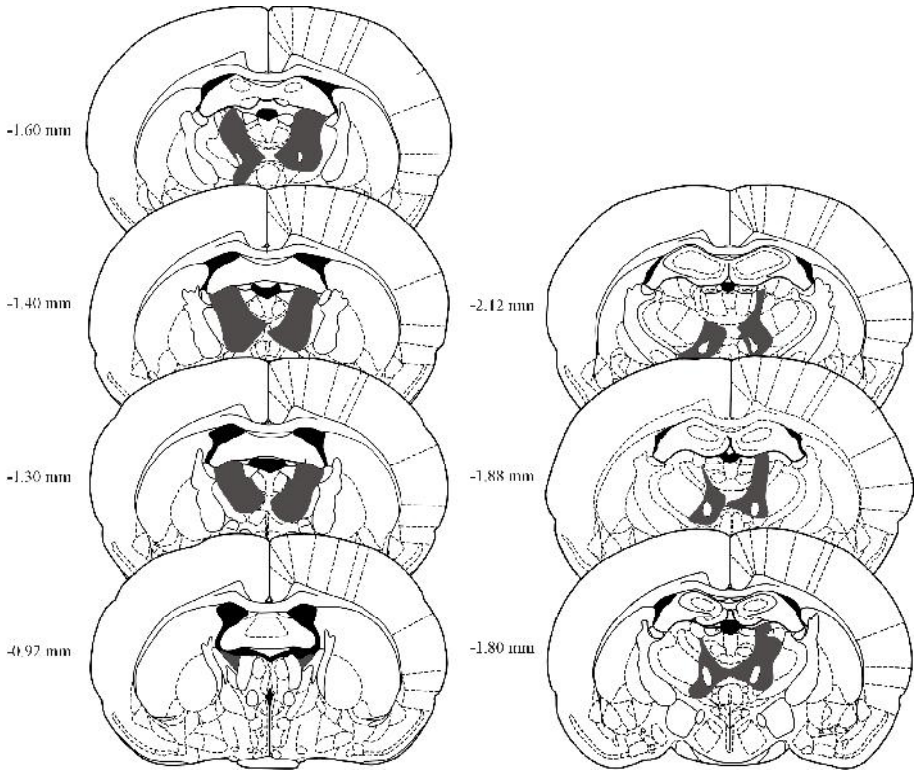

Figure 7.13. Rat #57 Sh-R. AT volume damage, 81%; LD volume damage, 0%.

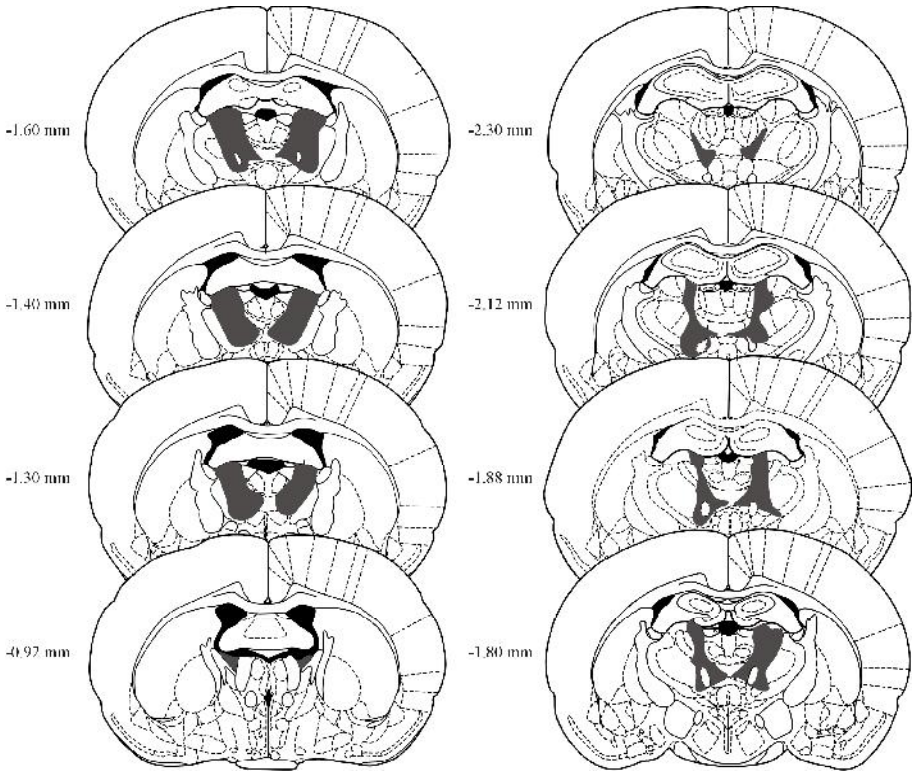

Figure 7.14. Rat #58 Ry-R. AT volume damage, 86%; LD volume damage, 1%.

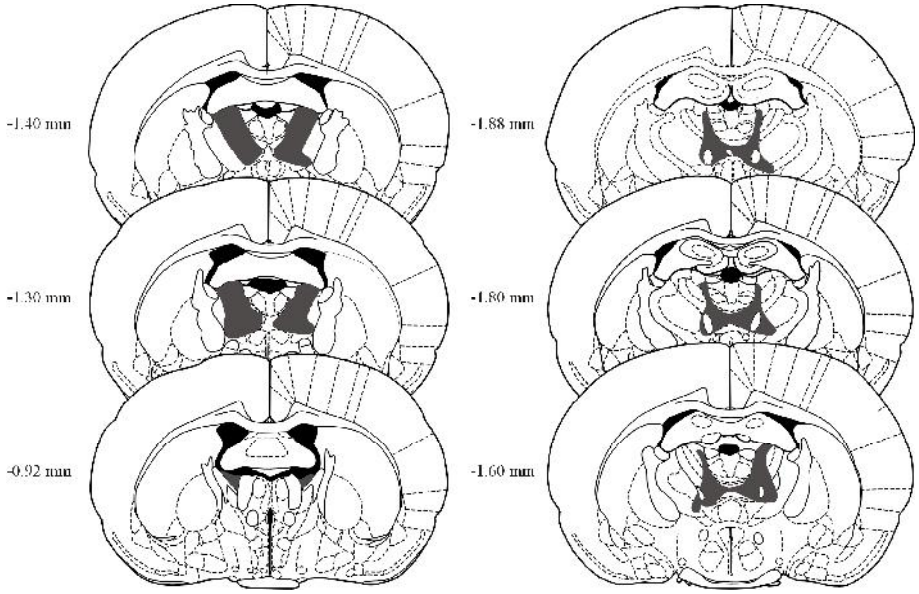

Figure 7.15. Rat #59 Sl-N. AT volume damage, 64%; LD volume damage, 0%.

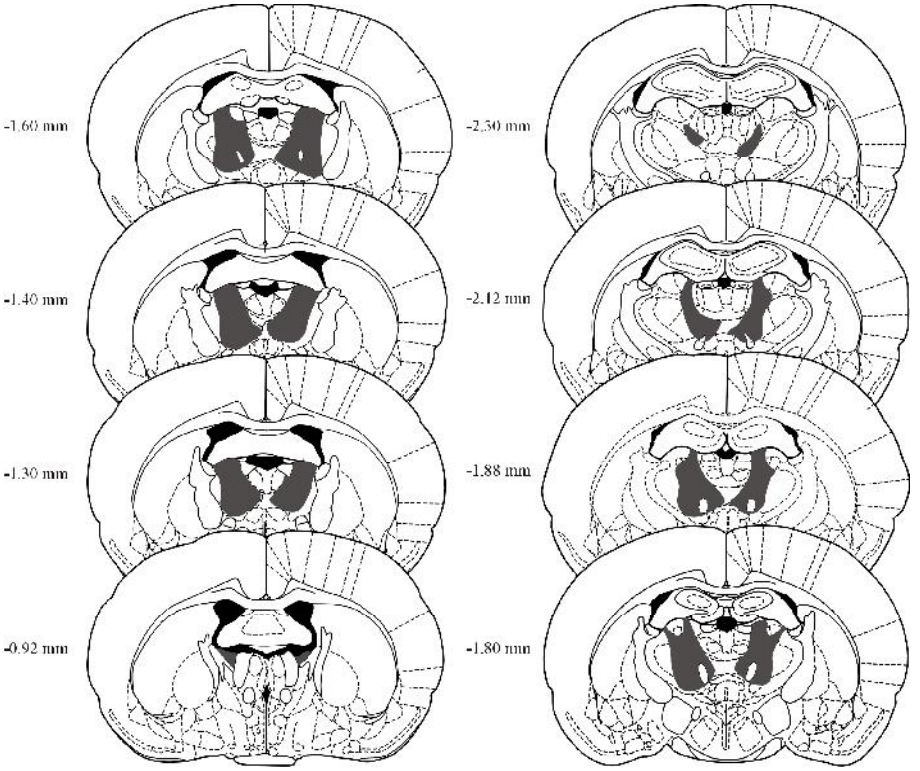

Figure 7.16. Rat #60 Qa-R. AT volume damage, 90%; LD volume damage, 1%.

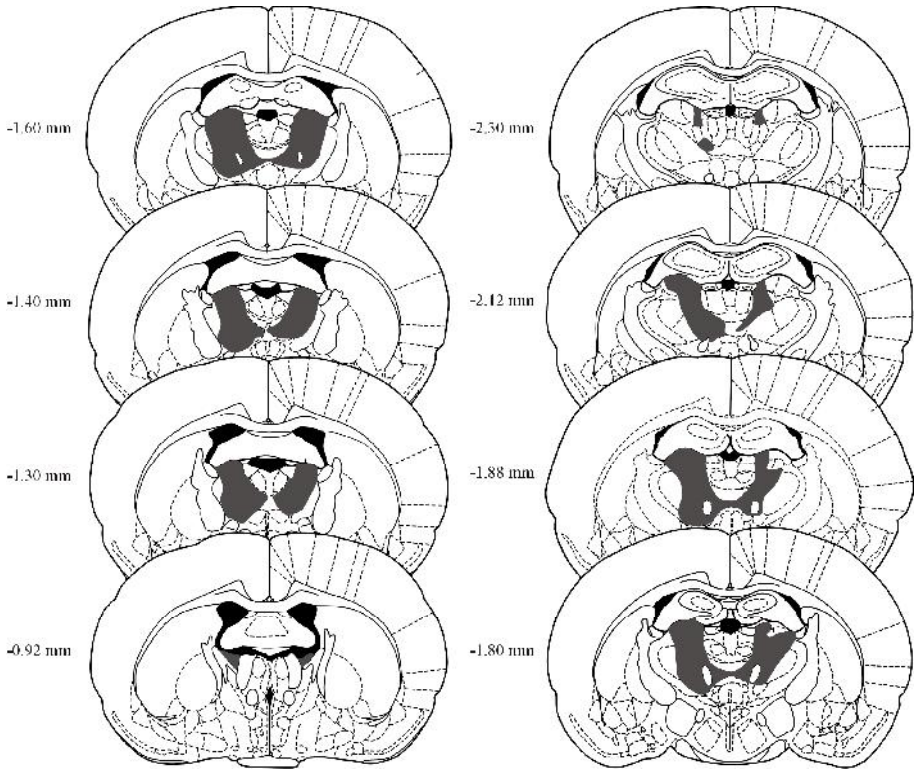

Figure 7.17. Rat #62 Ry-B. AT volume damage, 92%; LD volume damage, 8%.

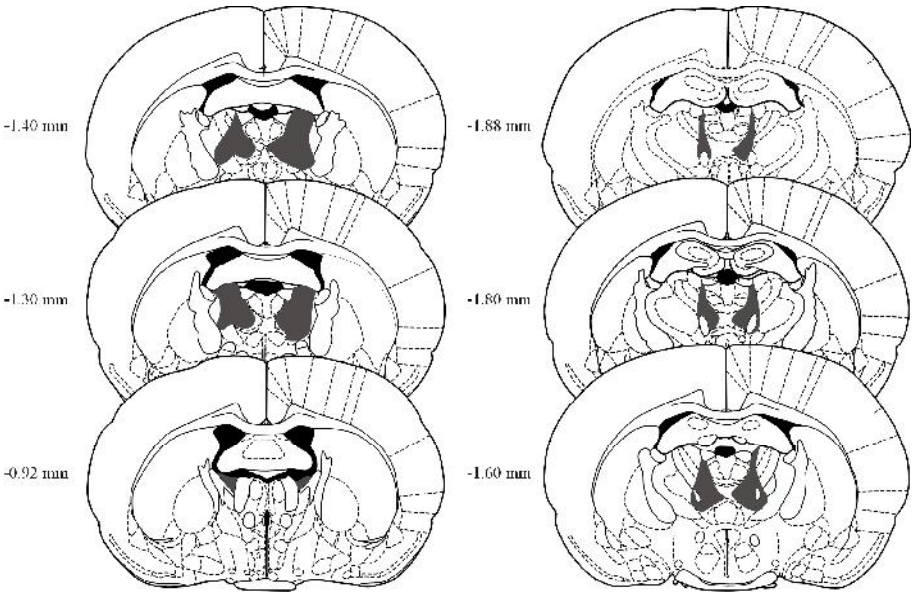

Figure 7.18. Rat #63 Ui-B. AT volume damage, 54%; LD volume damage, 0%.

7.3. Laterodorsal Thalamic Lesion diagrams

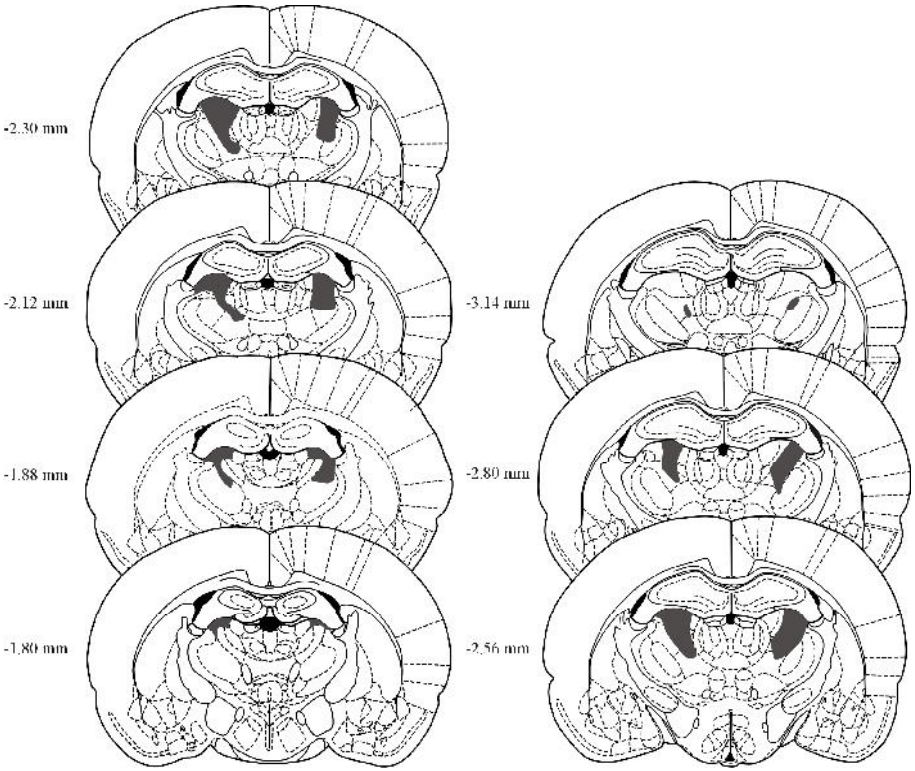

Figure 7.19. Rat #36 A-G. AT volume damage, 1%; LD volume damage, 37%.

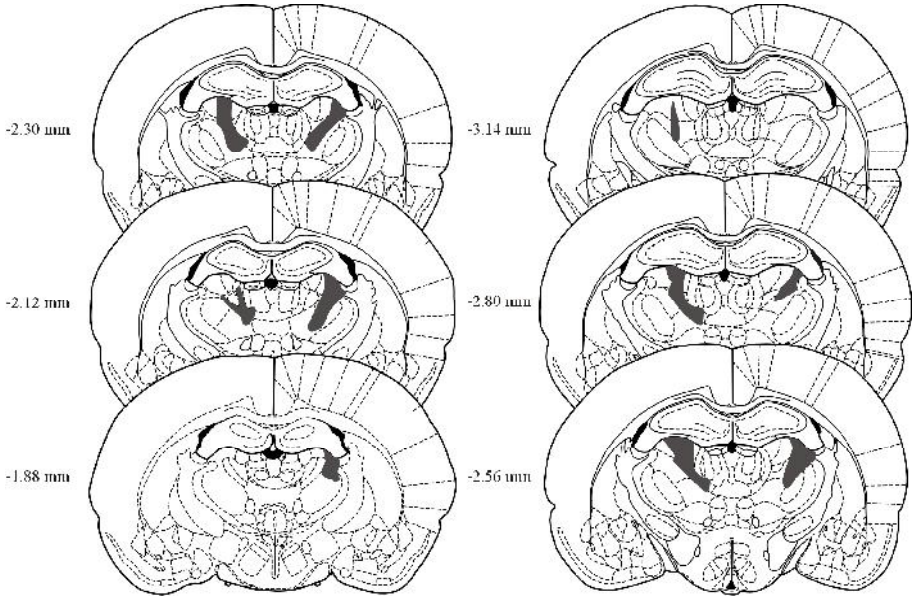

Figure 7.20. Rat #37 B-N. AT volume damage, 0%; LD volume damage, 19%.

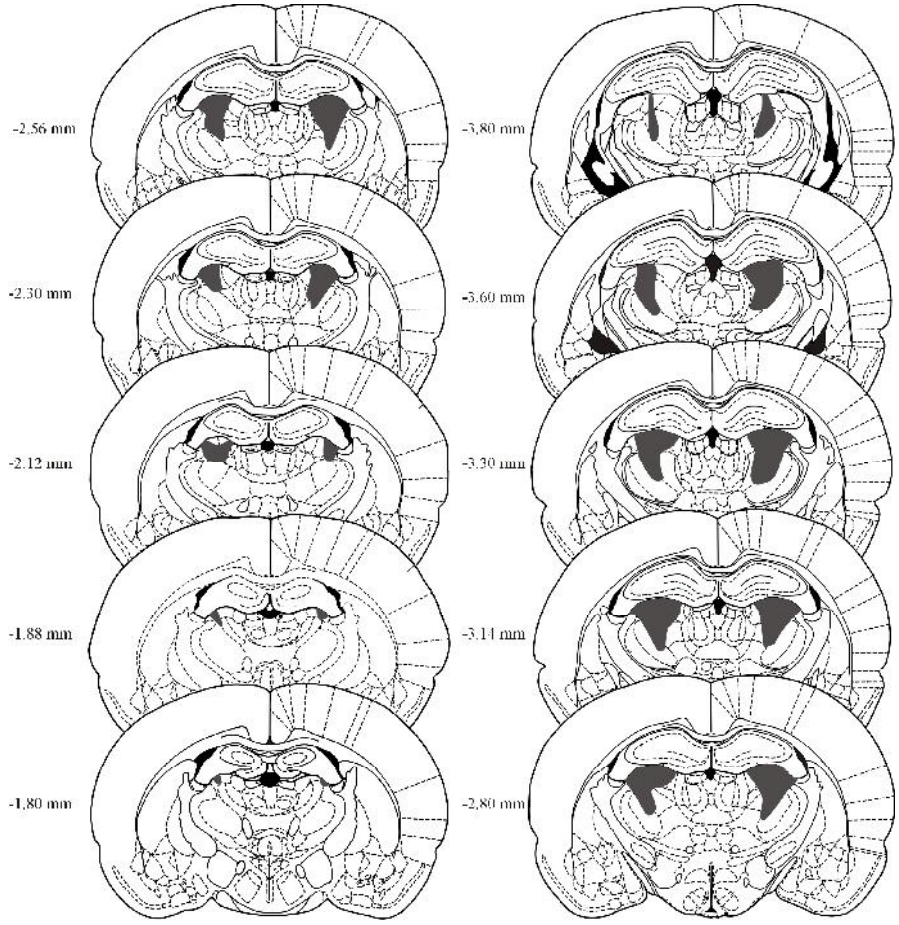

Figure 7.21. Rat #38 C-R. AT volume damage, 0%; LD volume damage, 76%.

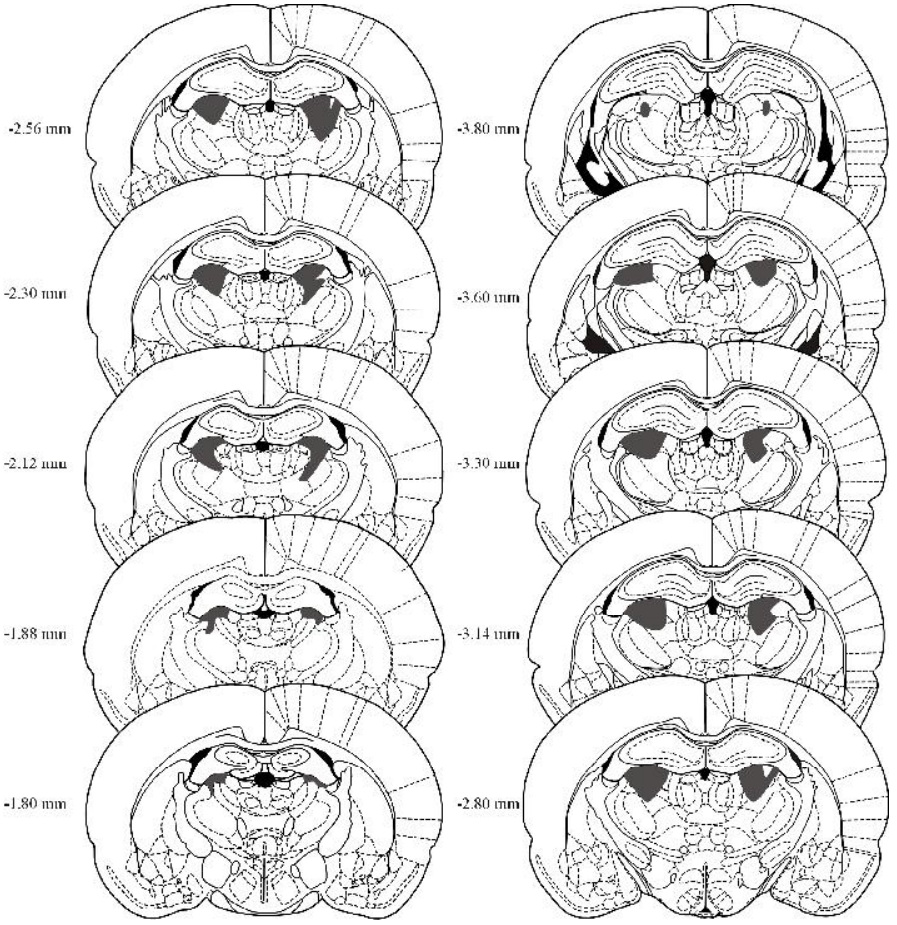

Figure 7.22. Rat #39 P-B. AT volume damage, 0%; LD volume damage, 80%.

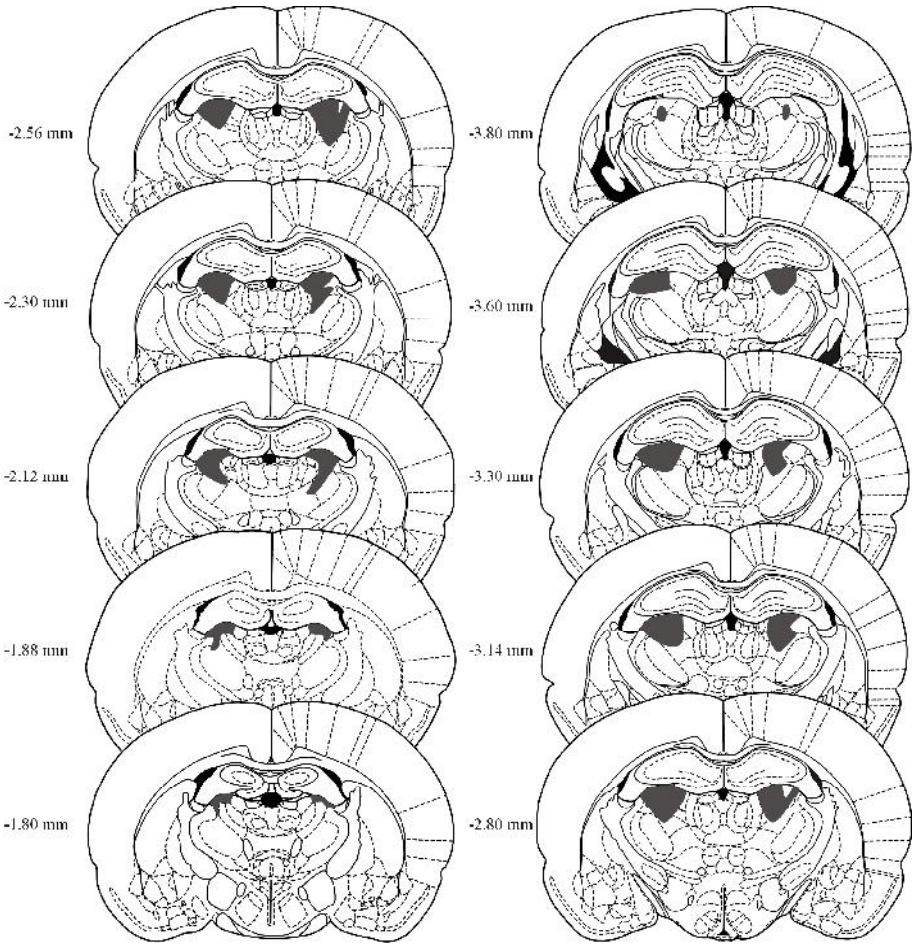

Figure 7.23. Rat #40 M-G. AT volume damage, 4%; LD volume damage, 50%.

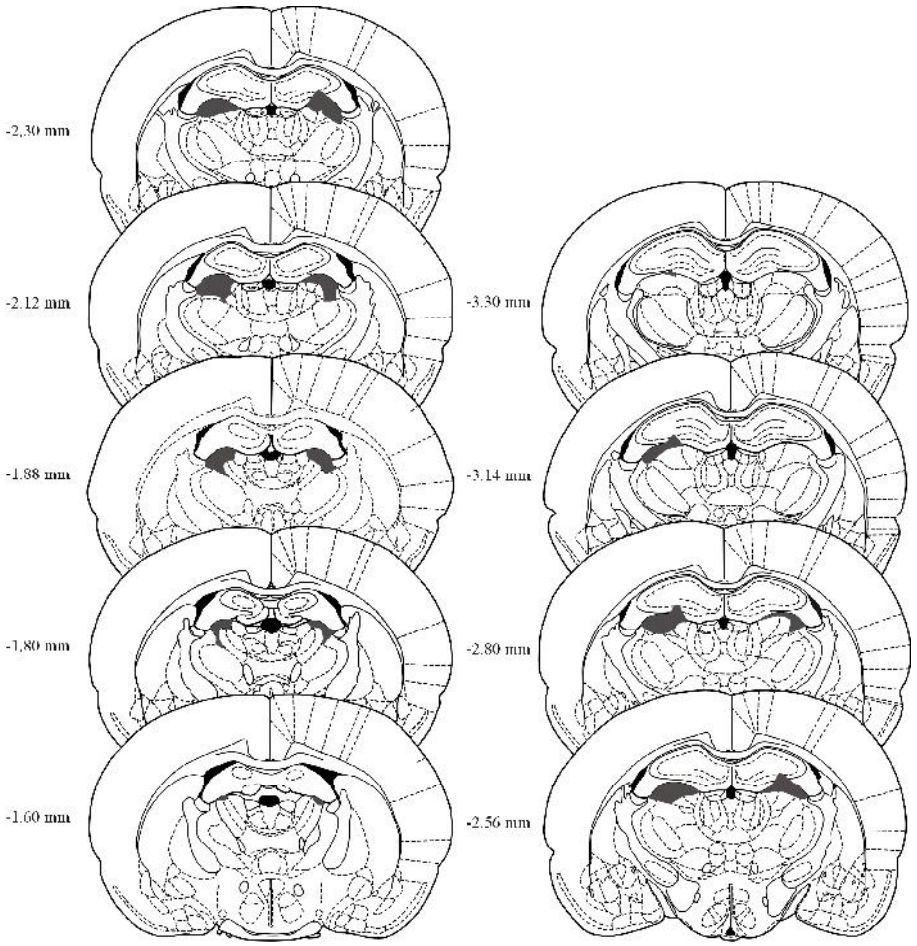

Figure 7.24. Rat #41 N-B. AT volume damage, 2%; LD volume damage, 40%.

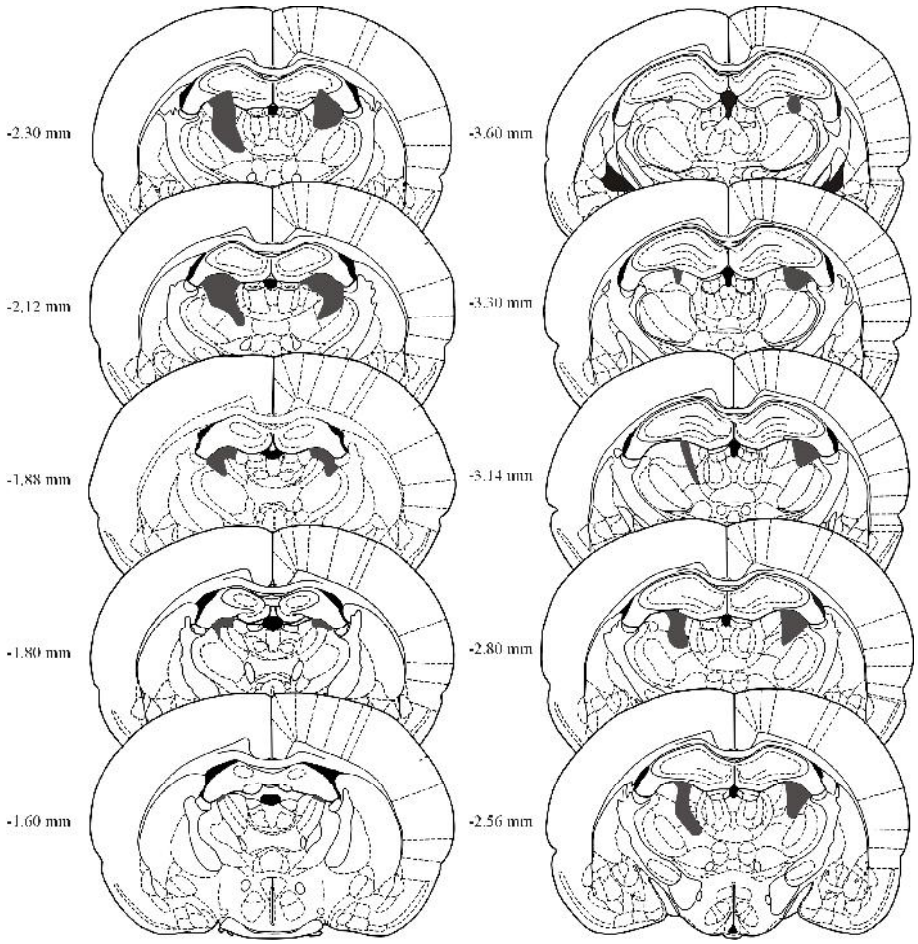

Figure 7.25. Rat #42 D-B. AT volume damage, 3%; LD volume damage, 48%.

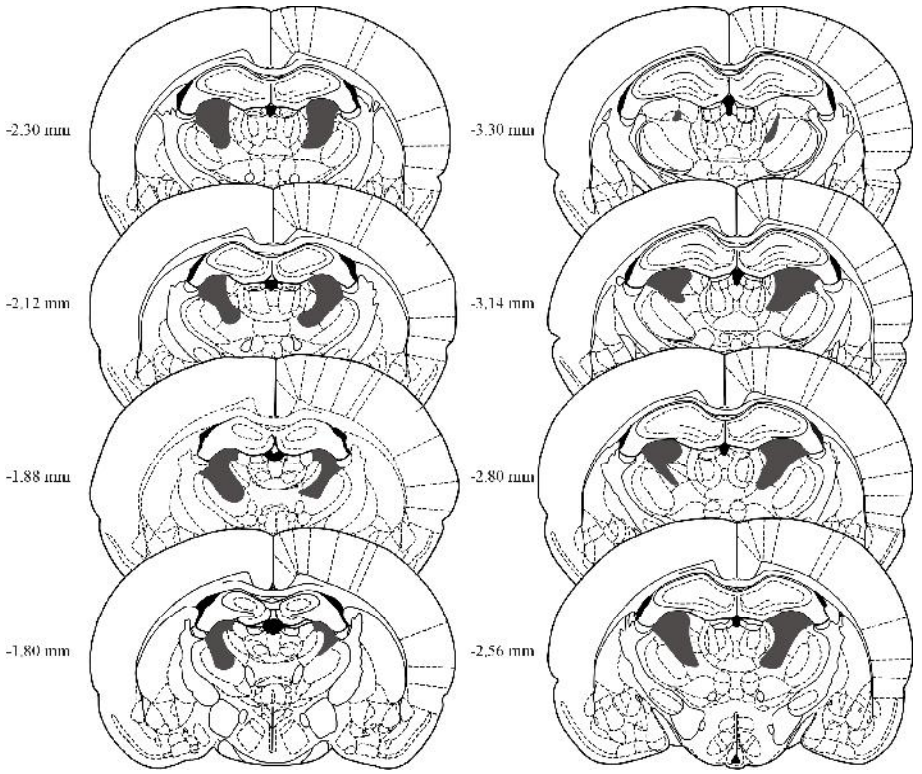

Figure 7.26. Rat #43 E-B. AT volume damage, 3%; LD volume damage, 66%.

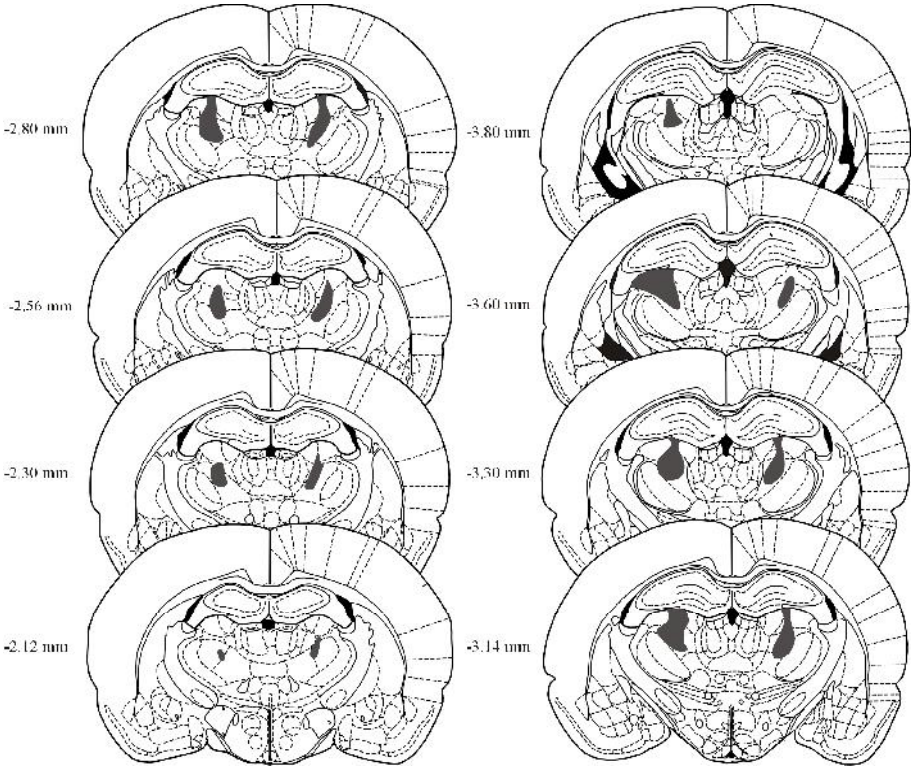

Figure 7.27. Rat #44 F-G. AT volume damage, 0%; LD volume damage, 23%.

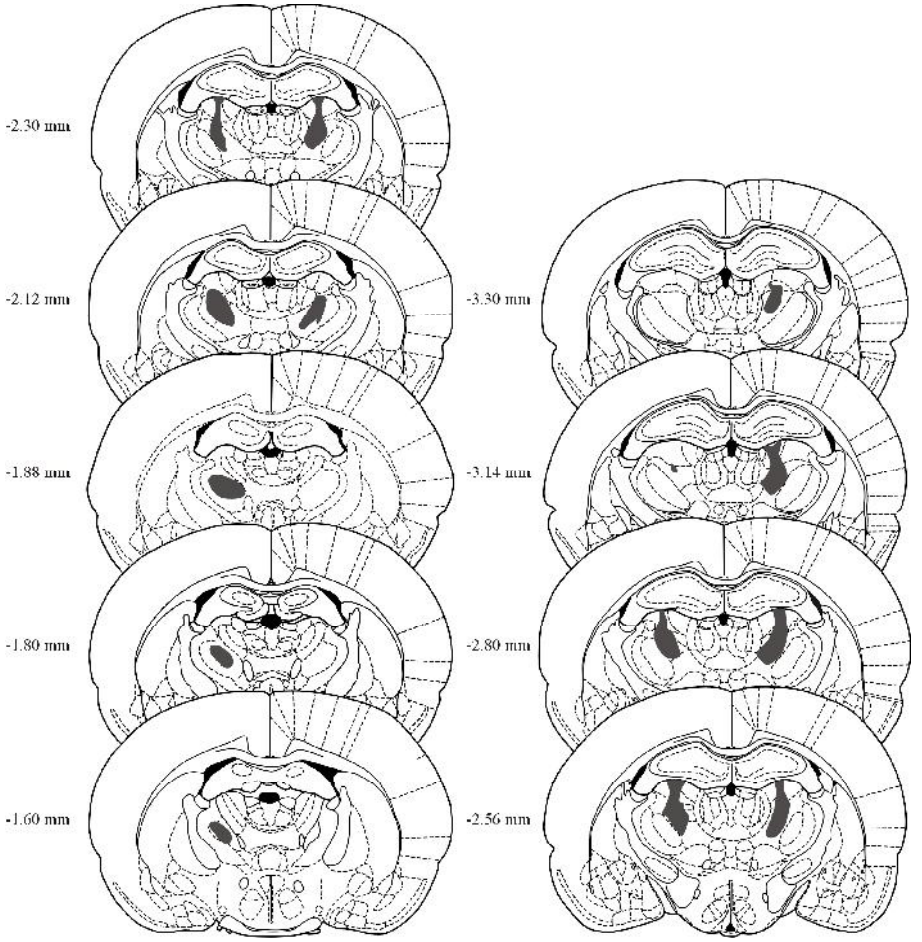

Figure 7.28. Rat #45 H-N. AT volume damage, 0%; LD volume damage, 18%.

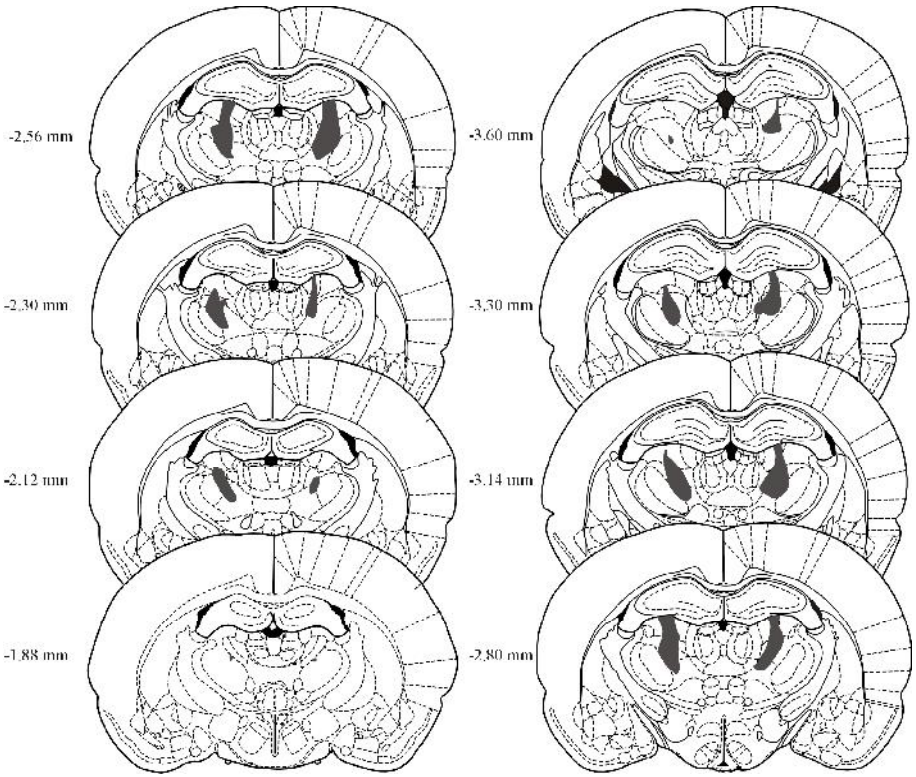

Figure 7.29. Rat #48 D-N. AT volume damage, 0%; LD volume damage, 20%.

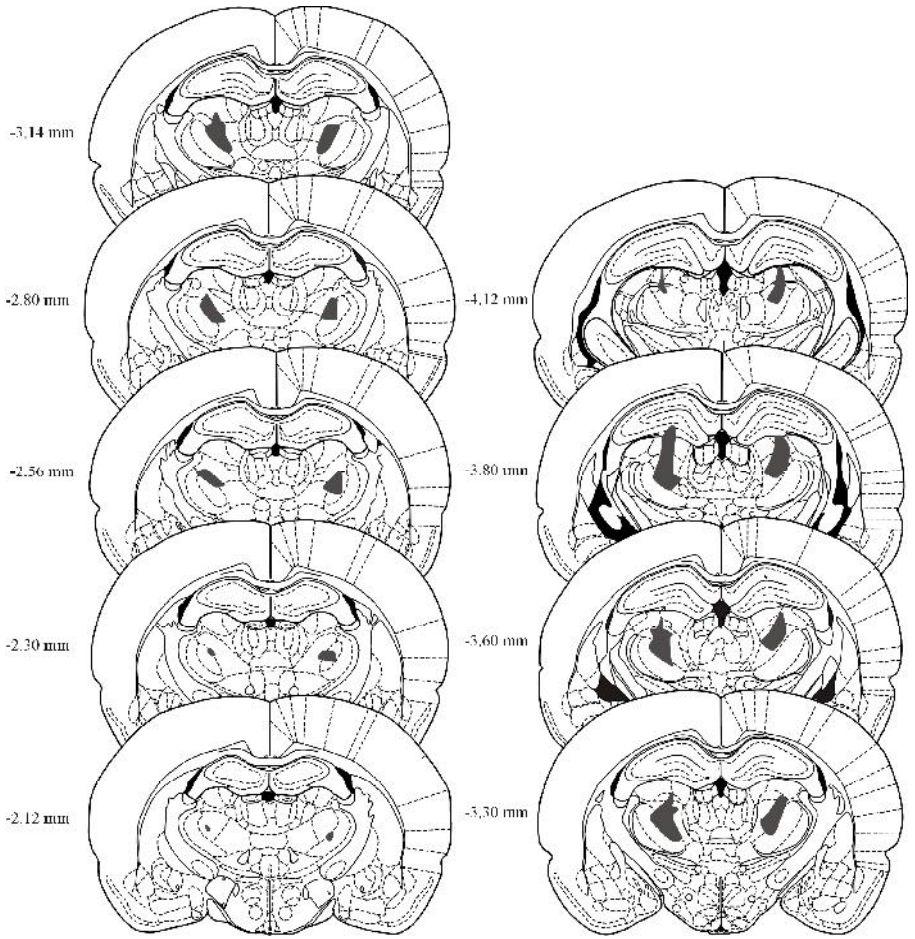

Figure 7.30. Rat #49 M-B. AT volume damage, 0%; LD volume damage, 2%.

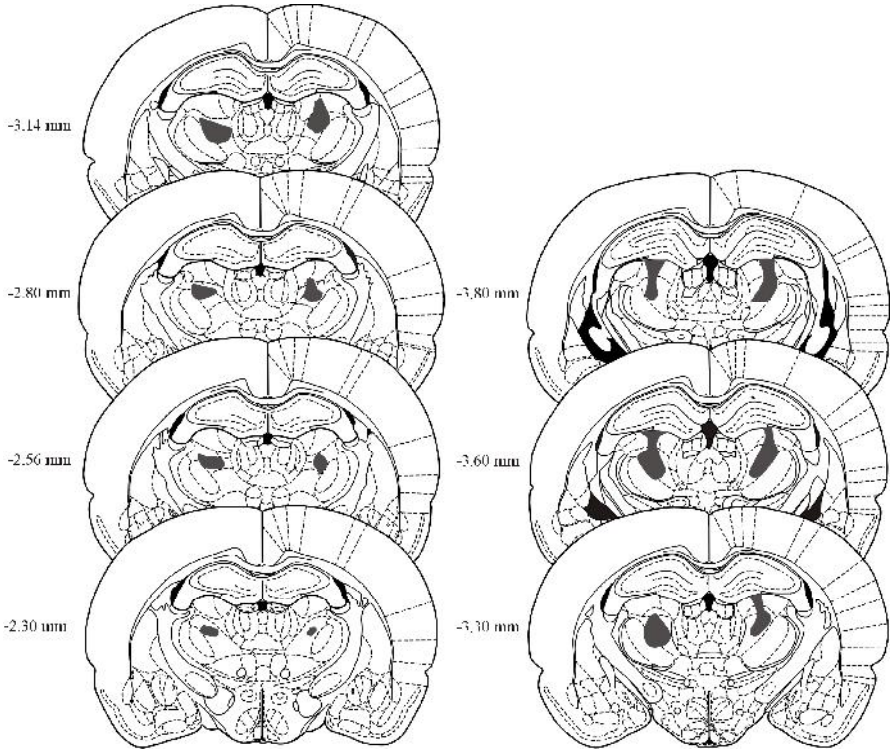

Figure 7.31. Rat #50 P-N. AT volume damage, 0%; LD volume damage, 7%.

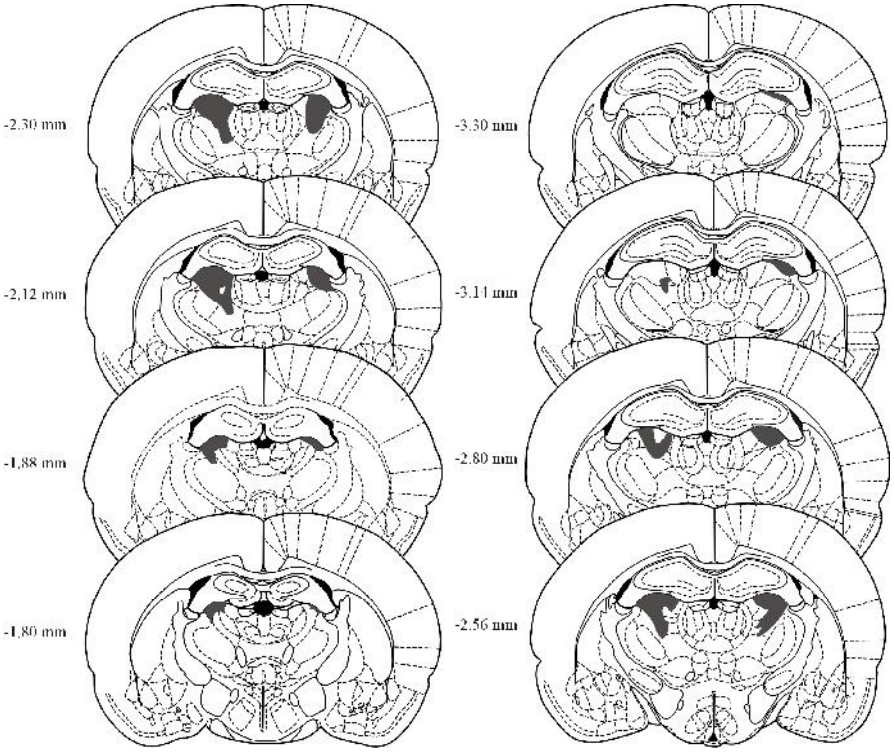

Figure 7.32. Rat #51 I-G. AT volume damage, 1%; LD volume damage, 46%.

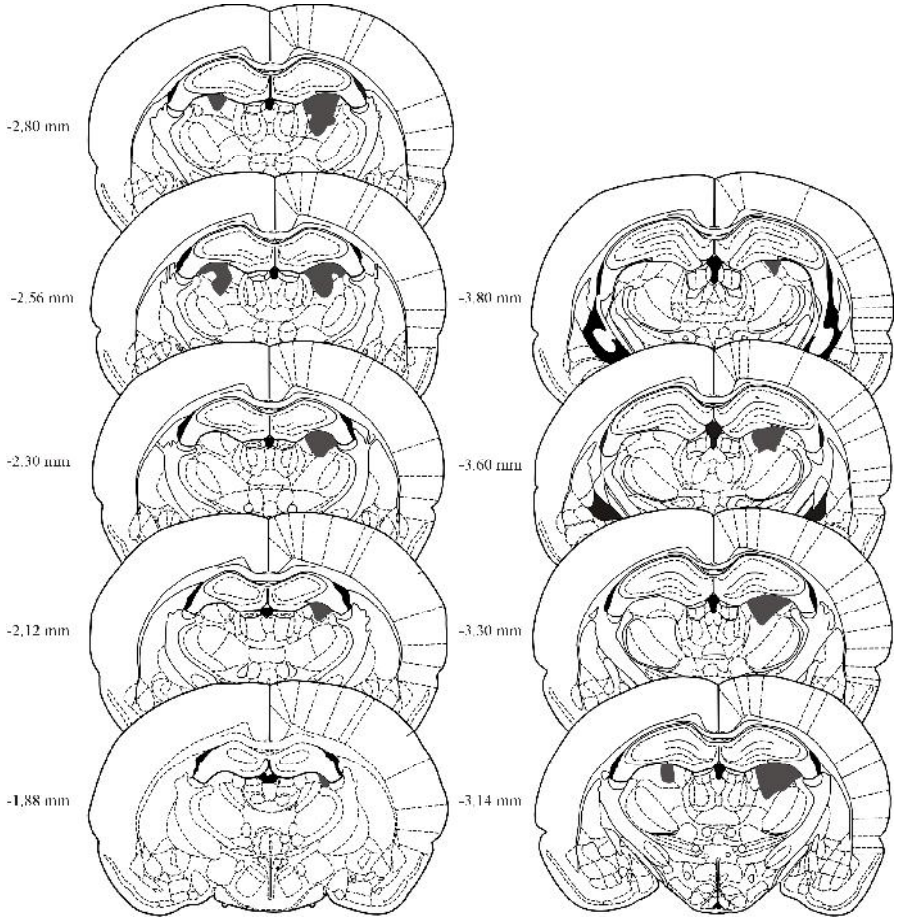

Figure 7.33. Rat #52 O-R. AT volume damage, 0%; LD volume damage, 48%.

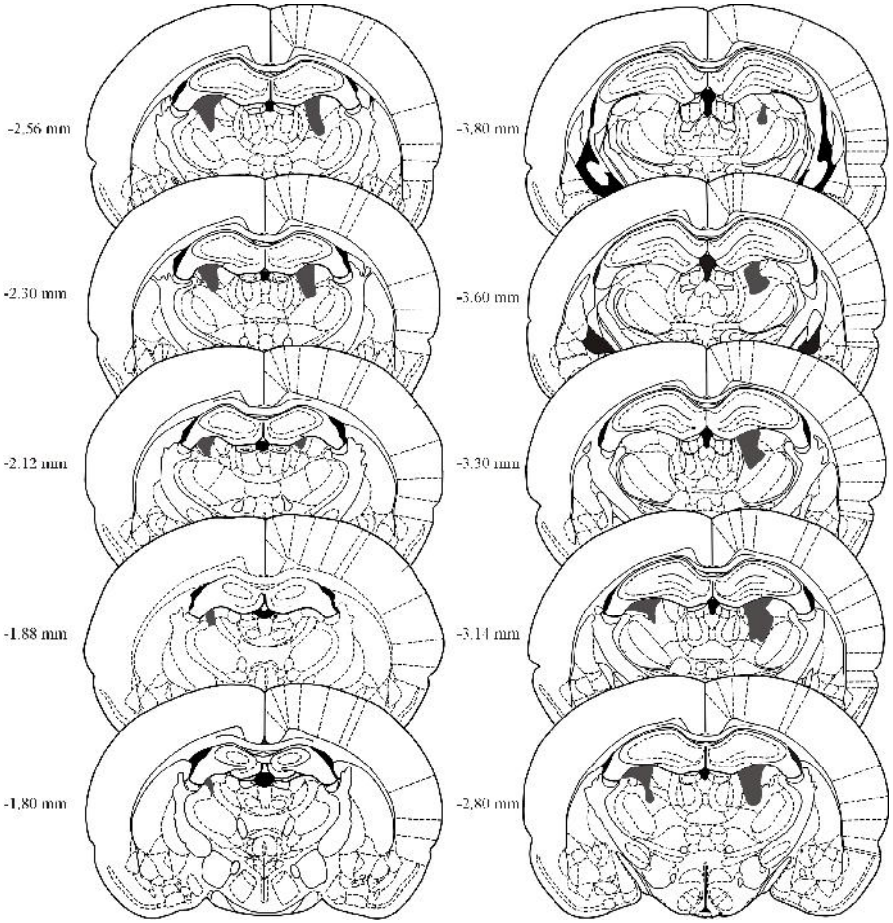

Figure 7.34. Rat #53 O-N. AT volume damage, 0%; LD volume damage, 40%.

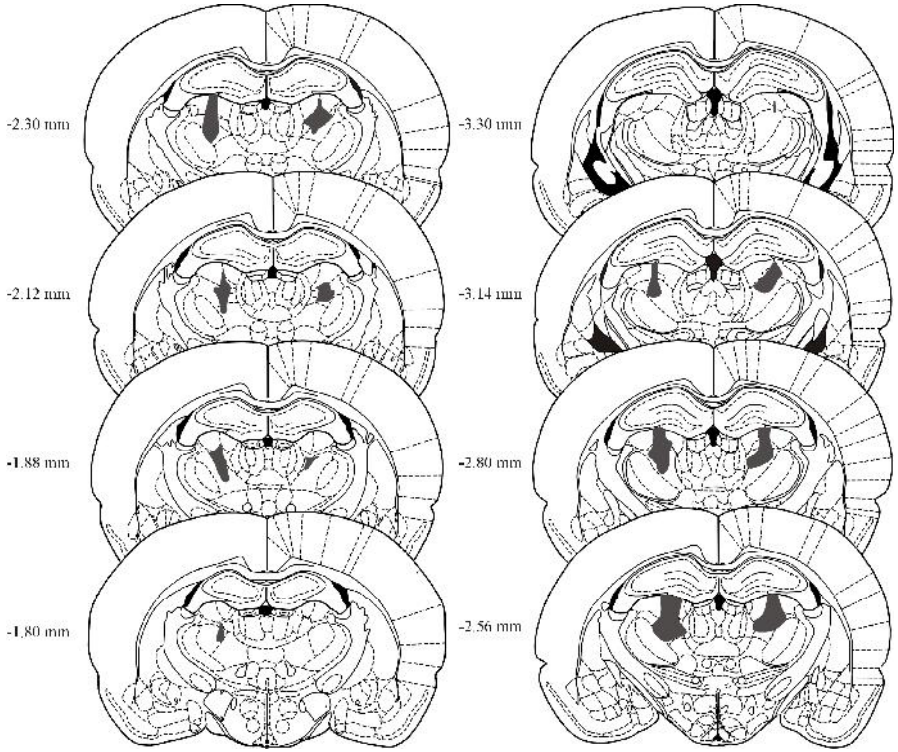

Figure 7.35. Rat #54 N-G. AT volume damage, 0%; LD volume damage, 23%.

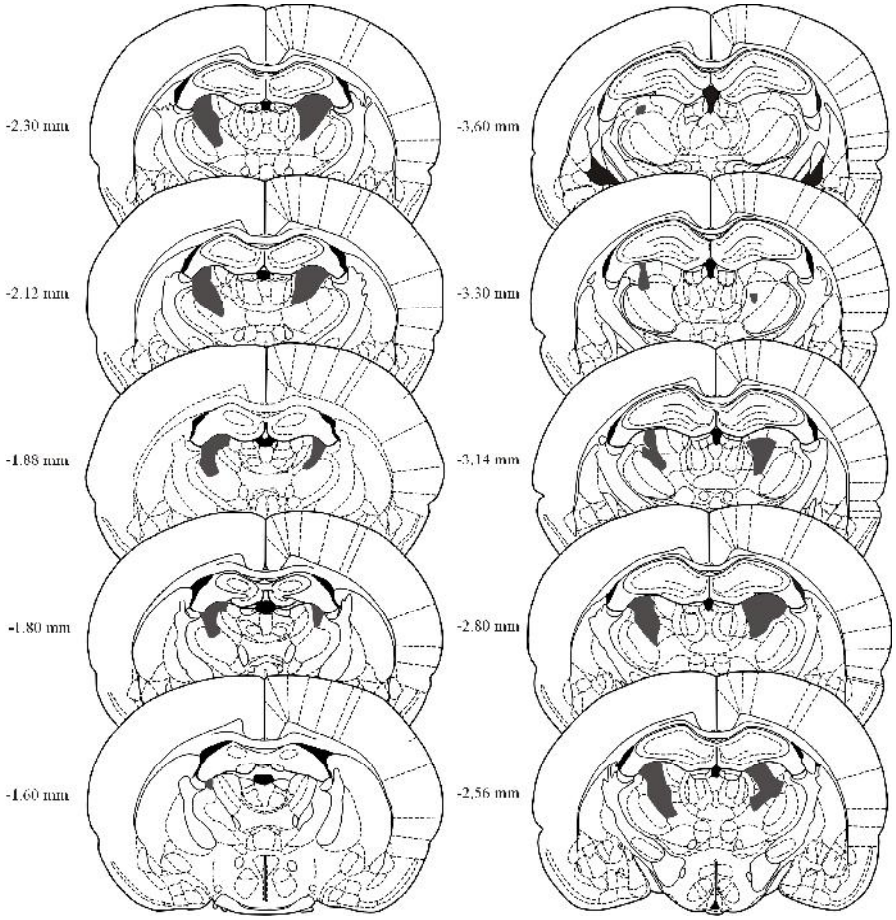

Figure 7.36. Rat #55 Uc-G. AT volume damage, 4%; LD volume damage, 47%.